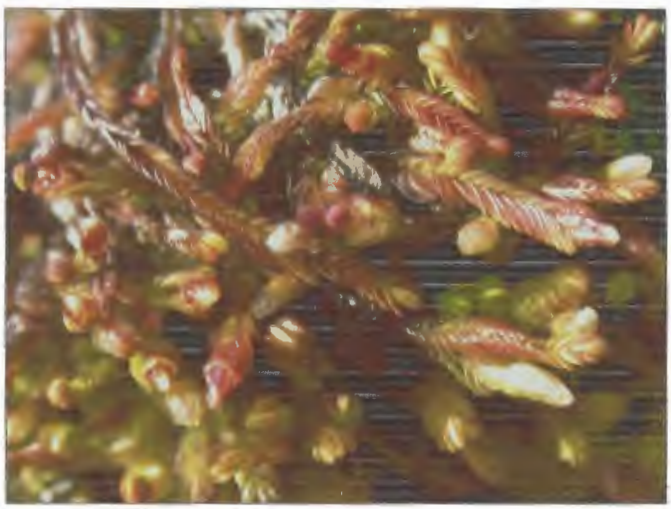
Palaeoclimatic Impacts on the Phylogeography of an Afro-montane Liverwort: *Jamesoniella colorata* (Lophoziaceae)



By

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Abstract

The mechanisms behind the high level of plant diversity and endemism observed in the Cape Floral Region (CFR) of South Africa have been the focus of many studies. Recently developed methods that employ DNA sequence data are making major contributions in reconstructing evolutionary histories of CFR species. Concurrently, palaeoenvironmental evidence is used increasingly to explain the impact of past climates on species ranges. This paper combines these two approaches by analysing the distribution of genetic diversity of the Afro-montane liverwort *Jamesoniella colorata* and associating its inferred evolutionary history with major palaeoclimatic trends in South Africa.

Liverworts are generally well-suited for phylogeographical studies because they often have low dispersal rates, broad geographical ranges and long evolutionary persistence. In addition, the high among-population diversity observed in *J. colorata* is conducive to the interpretation of significant historical events. The GIS-based bioclimatic envelope shows a strong correlation between potential habitat and the known distribution of *J. colorata* and indicates that sampling in this study was sufficient to make accurate phylogeographical inferences.

A combination of phylogeographical data and population genetics evidence suggests that populations of *J. colorata* in the Western Cape Province have experienced range contractions into upper-montane refugia and range expansions into lower altitudes in response to warming and cooling climatic trends, respectively. These range shifts have probably taken place throughout the Quaternary glacial-interglacials cycles, which are thought to have been influential in shaping modern patterns of diversity. In

an attempt to assign approximate dates to the two expansion events inferred for *J. colorata*, an average chloroplast mutation rate was applied to the *trn*L-F cpDNA mismatch distribution. The results roughly place the expansions within the last glacial period, demonstrating the general accordance of the phylogeographical and palaeoclimatic data.

The molecular work in this study also brought into question the taxonomic status of several specimens that showed distinctly divergent DNA sequences. Preliminary morphological inspection of the specimens revealed subtle but clear differences in leaf and stem anatomy that were once associated with *J. oenops*, a species synonymised with *J. colorata* in 1971.

Key words: phylogeography, *trn*L-F chloroplast DNA, *Jamesoniella colorata*, liverwort, South Africa, palaeoenvironment, bioclimatic envelope, mismatch distribution.

List of Tables and Figures

1. Introduction

1.1 Climate Change and Species Ranges

Climate changes during the Quaternary period (the last ~2.4 million years (Kukla, 1989)) have altered environmental conditions substantially, resulting in species range shifts and the restructuring of plant populations.

Worldwide, glacial-interglacial cycles have led to regular events of range expansion, contraction, speciation and extinction that have affected the distribution, abundance and genetic makeup of populations (e.g. Hewitt, 2000) Dramatic fluctuations of global temperature, ice volume and hydrological competence (Bush and Philander, 1999) have been imposed on regional parameters such as topography, latitude and oceanic and atmospheric circulation patterns (Hewitt, 2000). Range shifts have also been driven by species-specific physiological thresholds of temperature and precipitation tolerance, as well as life history parameters such as reproduction and dispersal ability (Walther et al., 2002).

Numerous studies have attempted to reveal the mechanisms behind the exceptional biodiversity found in the Cape Floristic Kingdom (CFR) of South Africa (Linder, 2003; Midgley et al., 2003). Dominated by the Fynbos and Succulent Karoo biomes, the CFR surpasses most tropical floras in species richness (Cowling et al., 1992) and has a high level of endemism usually only observed in island ecosystems (Linder, 2003).

Physical factors such as edaphic conditions, complex topography, fire regimes and climate change have all been explored as speciation drivers in the CFR and other

Mediterranean-type ecosystems (e.g. Cowling, 1987; Cowling et al., 1992; Linder, 2003; Midgley et al., 2003; Richardson et al., 2001). Recently, efforts at reconstructing the evolutionary histories of CFR species have integrated species ranges into palaeoclimatic modelling techniques (e.g. Midgley et al., 2001). This type of approach is promising, but can be improved upon by including molecular evidence on Cape flora population histories.

The recently introduced molecular method explored in this paper uses genealogical and geographical information on DNA sequence data to infer population histories (Templeton et al., 1995). Because 'phylogeography' can be applied to all taxa, and be compiled to reflect changes in plant communities, it has become a popular means of examining biome-level evolution. This study demonstrates the versatility of phylogeography, by attempting to link the evolutionary history of a CFR species to palaeoclimatic trends in South Africa.

1.2 Review of the South African Palaeoclimatic Record

A synthesis of major South African palaeoenvironmental data sources and their inferred climatic signals is presented in this section. This review of climatic change, following the temporal scale of regional proxy data, focuses primarily on the period from 130,000 BP to present. However, climate changes of the last interglacial-glacial cycle can broadly be extrapolated over at least the past ~1 million years (deMenocal, 1995).

To facilitate regional climate comparisons in this study, South Africa is divided into three regions based on modern precipitation seasonality. Figure 1 illustrates the

placement of the Winter Rainfall Zone (WRZ), the Summer Rainfall Zone (SRZ) and the Year-round Rainfall Zone (YRZ), which are delimited by a percentage winter rainfall gradient after Cockcroft et al. (1987).

Variations in rainfall patterns in southern Africa during the Quaternary period are thought to have resulted from episodic displacements of atmospheric circulation systems. Presently, in the WRZ, periods of increased precipitation occur as the result of seasonal equatorward shifts of the circumpolar westerlies and their related frontal systems. Conversely, drier conditions in the WRZ occur when the westerlies migrate south of the continent and stronger easterly flow from the Indian Ocean brings summer precipitation in the SRZ (Tyson, 1986). These general precipitation mechanisms have been extrapolated into the past to explain broad-scale climate changes during the Quaternary, with winter and summer precipitation regimes being applied to glacial and interglacial periods respectively (Cockcroft et al., 1987).

1.2.1 The Last Interglacial – Last Glacial Maximum (~130,000-22,000 BP)

The Last Interglacial and early glacial periods are poorly represented in southern African palaeoclimatic archives. General global trends from stable isotope evidence in polar ice cores (shown in figure 2) indicate that the peak of the Last Interglacial occurred from ~130,000 to 115,000 BP (Petit et al., 1999). Temperatures are inferred to have been approximately 2°C warmer than present (Guiot et al., 1989) and the westerlies in Africa are thought to have shifted poleward, decreasing the size of the WRZ (Cockcroft et al., 1987).

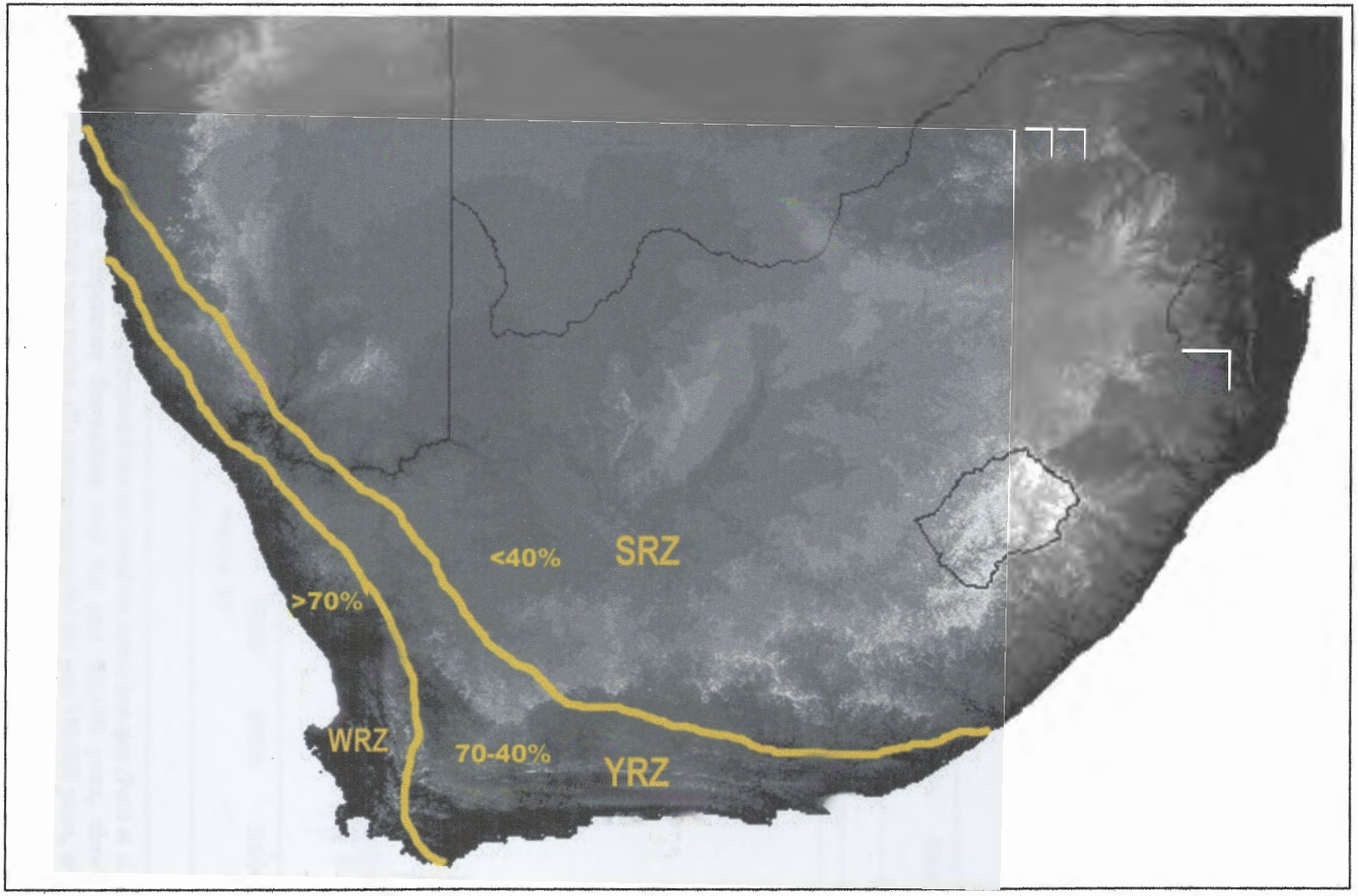
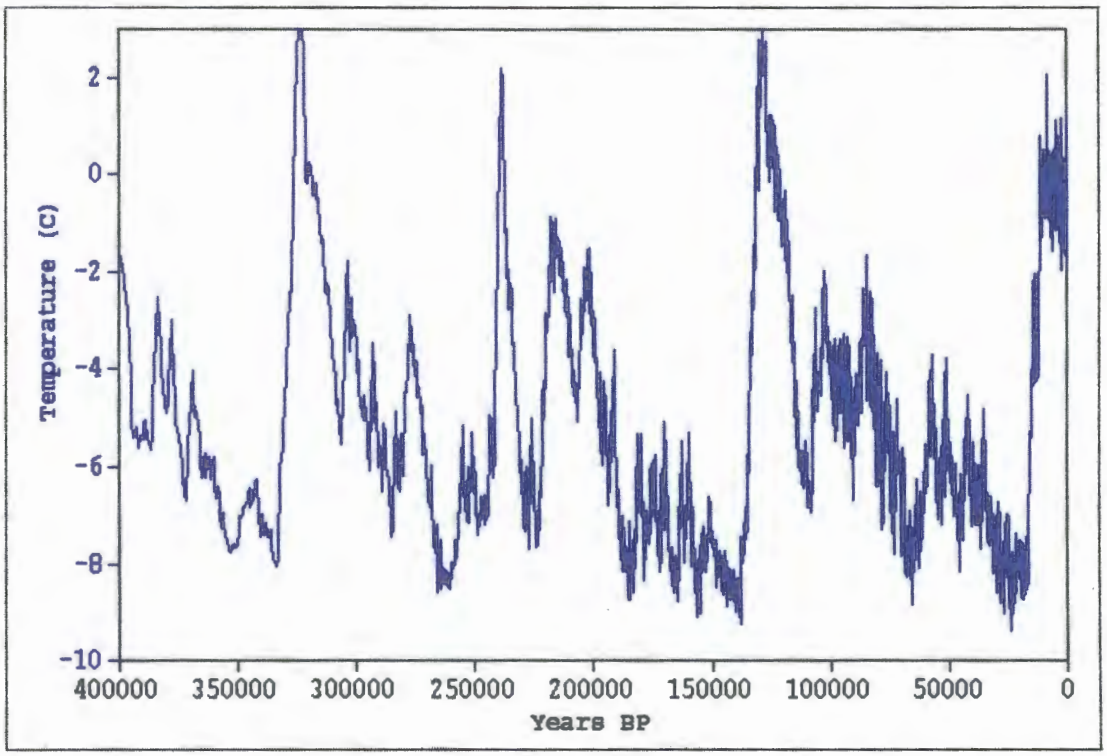


Figure 1. Southern African rainfall seasonality gradient along percentages of winter rainfall after Cockcroft et al (1987). The Winter Rainfall Zone (WRZ) is defined as having over 70% of its total annual rainfall in winter. The Year-round Rainfall Zone (YRZ) has 70-40% of its rainfall in winter, while in the Summer Rainfall Zone (SRZ), less than 40% of rainfall occurs in winter.

(A)



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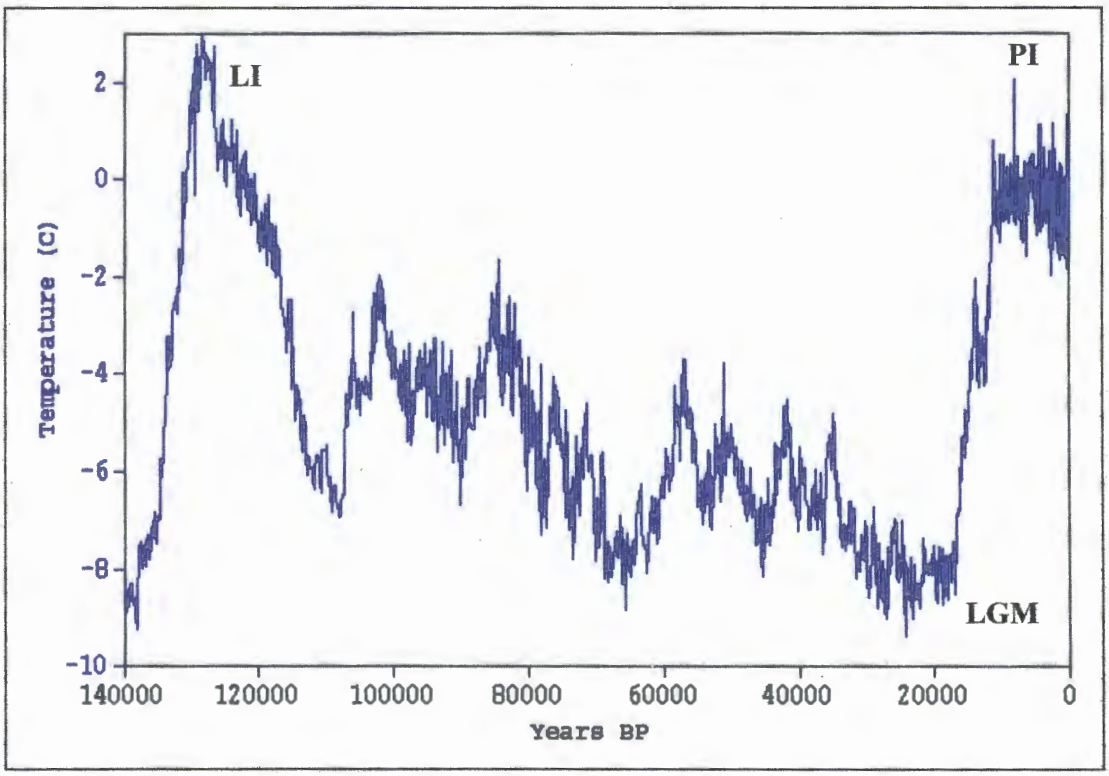

Figure 2. Vostok ice core temperature curves based on stable isotopes (Petit et al. 1999). (A) Inferred global temperature fluctuations over the past 400,000 years, showing glacial-interglacial 100,000 year cycles. (B) Isotope record for the past 140,000 years, with evidence of the Last Interglacial (LI), the Last Glacial Maximum (LGM) and present interglacial period (PI). The Holocene Altithermal is not seen on this record.

Significant global cooling began by ~115,000 BP and extended through the Last Glacial Maximum (LGM) to ~18,000 BP (Petit et al., 1999). Over this long period, global temperatures fluctuated in the range of ~2-10°C cooler than present day (Guiot et al., 1989; Ponel, 1995), with evidence from southern Africa suggesting maximum temperature depressions on the order of ~5-6°C (Stute and Talma, 1997). Concurrently, the westerlies intensified and expanded, increasing the area of the WRZ and bringing more moisture to the southwestern Cape (Cockcroft et al., 1987).

1.2.2 LGM (~22,000 – 18,000 BP) to Present

The late Quaternary palaeoenvironmental record of southern Africa is based on numerous proxy data sources such as pollen, charcoal, and fossil data. Detailed scenarios of climate change must be chronologically constrained, and thus much of significant evidence is restricted to the last ~40,000 years, the limits of radiocarbon dating. Within this period, the LGM and the Holocene Altithermal (HA) are considered to be the coldest and warmest phases respectively (Partridge et al., 1999).

1.2.2.1 Last Glacial Maximum (LGM)

It has been suggested that during the LGM most of South Africa, including the SRZ and the YRZ experienced a cooler and drier climate than present (Meadows and Baxter, 1999; Tyson, 1999). From this latter region, records from Boomplaas Cave, located in the foothills of the Swartberg range, are interpreted as being strongly indicative of colder and drier conditions at the LGM (Deacon and Lancaster, 1988).

Data from multiple sources in the WRZ agree that LGM temperatures were cooler than present (Meadows and Baxter, 1999). Scott (1994) points to a lowering of vegetation belts in the Cederberg range around this time as further evidence of cooler conditions. The important difference in the WRZ, however, is the associated increase in rainfall. Pollen evidence from Elands Bay Cave strongly supports a cooler and wetter LGM, with reduced seasonality of rainfall (Parkington et al., 2000).

It is important to note that although the palaeoenvironmental evidence has been interpreted as indicating that the YRZ and WRZ experienced diametric trends during the LGM, with the former being more humid, and the latter more arid, these trends are in relation to present day conditions. Today, the seasonality of rainfall in WRZ and YRZ has resulted in the establishment of very different vegetation communities, and what is seen as a drying trend in the YRZ may in fact simply have been an increase in seasonality as the WRZ expanded eastward (Brian Chase pers. comm.).

1.2.2.2 Holocene Altithermal (HA)

The period between the end of the end of LGM and the beginning of the present interglacial (the Holocene, ~10,000 BP to present) is characterised by steady and dramatic warming over much of southern Africa (Cockcroft et al., 1987). The HA is a thermal peak that occurred in South Africa between approximately 8,000 and 4,000 BP, with an increase 1-2°C above average Holocene temperatures (Partridge et al., 1999) and 6-8°C above LGM averages.

Similar to the LGM, the accompanying precipitation changes of the HA were not consistent throughout South Africa. Evidence from a variety of sources in the SRZ suggests moister conditions than present during the HA (Avery, 1993; Partridge et al., 1990; Scott, 1993), while pollen records in the southwestern Cape indicate that the WRZ was drier (Baxter, 1989; Meadows and Baxter, 2001), a phenomenon attributed by Iriondo (1999) to the 5 degree southward shift of the westerlies.

Drier conditions have also been suggested for the YRZ. Samples from Boomplaas Cave, dated to ~7,300 BP, indicate warm and dry conditions, with notable periods of drought (Scholtz, 1986).

1.2.2.3 Late Holocene

Climatic conditions since the HA have fluctuated around a slightly cooler mean temperature, though with less pronounced variations compared to the LGM and HA (Cockcroft et al., 1987).

Continuous isotope data from the last 5,000 years at Cango Cave in the YRZ show two brief cooler periods at 4,700 BP and 3,200 BP, with an intervening period of mild conditions (Tyson, 1999). Also evident are the globally recognised Medieval Warm Epoch (900 to 1300 AD) and Little Ice Age (1300 to 1800 AD) (Tyson et al., 2001).

In terms of precipitation, an increase in summer rainfall occurred around 6,000-5,000 BP, marking the formation of the year-round rainfall pattern in the YRZ (Avery, 1993). In the WRZ, organic sediments in Verlorenvlei suggest greater moisture

availability in the past 5,000 years (Meadows and Baxter, 1999). Higher frequencies of Ericaceae and Restionaceae pollen in Cecilia Cave also indicate predominately wetter conditions around 3,500 BP (Baxter, 1989). These data confirm the hypothesis that cooler temperatures in the WRZ have been associated with wetter conditions (Cockcroft et al., 1987)

To simplify late Quaternary climatic conditions in South Africa, a table is presented below outlining the predominant trends of each region during the major climatic periods.

Table 1. A summary of major late Quaternary palaeoclimatic trends for each region. Descriptions are relative to present conditions except for those in the Late Holocene, which are compared to HA conditions.

Region	LGM	HA	Late Holocene - present
WRZ	Cooler and wetter	Warmer and drier	Cooler and wetter than HA
YRZ	Cooler and drier	Warmer and drier	Cooler and wetter than HA
SRZ	Cooler and drier	Warmer and wetter	Cooler and drier than HA

1.3 Reconstructing Population History

How can we correlate climatic history with contemporary patterns of genetic variation in populations? It is first necessary to determine what evolutionary processes may have led to present day genetic structure by employing a variety of methods that estimate population history. In this study, a combination of traditional population genetic and coalescent-based methods is used to present historical evidence on populations of *J. colorata*.

1.3.1 Population Genetic Methods

Traditional population genetics methods rely on the statistical treatment of effective population sizes (Harding, 1996). Wright's *F* statistics, including the frequency of diversity among populations (*F*st), is widely used to estimate micro-evolutionary parameters such as gene flow and genetic drift, that are considered key evidence in the reconstruction of population histories (Excoffier et al., 1992). Patterns of genetic partitioning in populations can also support hypotheses on historical events. However, if used exclusively, population genetic methods often fail to detect different models of population structure and therefore may imply false population histories (Templeton et al., 1995).

1.3.2 Coalescent-based Methods

More sophisticated methods rely on the coalescent, a model of lineage sorting that runs backward in time to common ancestors (Emerson et al., 2001). Its foundation was laid by population geneticists in the 1980s and has since been refined by advances such as the concept of neutral gene evolution and molecular DNA sequencing (Harding, 1996).

Central to coalescent theory are gene genealogies, or trees of allelic descent, with extant individuals positioned as 'branch tips' that coalesce back to common ancestor 'nodes' (Avise, 2000). Because they display allele divergence over time, gene genealogies allow inferences to be made on historical and demographic population processes, such as population growth, genetic drift and migration, within a temporal context (Schaal and Olsen, 2000). Gene genealogies can also be spatially informative,

as alleles can be sampled across species distributions or particular geographic barriers (Avise, 2000).

1.3.2.1 Mismatch Distributions

Graphs of pairwise nucleotide differences between samples, or mismatch distributions, reflect the shape of gene genealogies (Rogers and Harpending, 1992) and therefore can be used to estimate past population growth (Schneider and Excoffier, 1999). If the DNA region mutation rate is known, the pairwise peaks of expansion events can be dated (Avise, 2000), although the accuracy of such estimates is dependent on the reliability and heterogeneity of the mutation rate. However, for practical purposes, mismatch distributions are useful in providing estimates of past demographic change in populations (Schneider and Excoffier, 1999).

1.3.3 Phylogeography

The flexibility and strength of coalescent theory is evident in phylogeography, the study of population level genetic diversity through space and time. Phylogeographical studies investigate the distribution of allele variation in an explicit geographical context (Schaal and Olsen, 2000) in order to elucidate the biological factors that have led to contemporary genetic patterns within and among populations.

1.3.3.1 Nested Clade Analysis

One of the more powerful phylogeographical methods uses a nested series of branches from an allele tree to infer population structure and history. Templeton and Sing

(1993) developed the nested clade analysis (NCA) to "test the null hypothesis of no geographical association of haplotypes, test the hypothesis that significant associations are due to restricted gene flow, and identify patterns of significant association that are due to historical events". Such patterns can result from restricted gene flow, population fragmentation, range expansion or colonization (Templeton et al., 1995).

The framework of NCA is a hierarchical haplotype network based on mutational steps of a variable allele, where the age of haplotypes decreases from the centre to tips. Estimated clade distances (the geographical spread of a clade (D_C)) and nested clade distances (the relative geographical distribution of a clade (D_N)) of the haplotypes are compared using a Monte Carlo likelihood procedure (Posada et al., 2000) and significant results are matched against the expected patterns of the four possible historical events. In this respect, NCA allows the interpretation of historical processes at all clade levels, so that geographical associations can be correlated with local events.

Although the NCA is considered more advanced than traditional models because it incorporates geography and offers a wider array of historical explanations, it does have several limitations. It requires comprehensive and often time consuming sampling across study areas in order to accurately detect significant patterns (Cruzan and Templeton, 2000) and does not assess confidence estimates in its interpretations. To emphasise this shortcoming, Knowles and Maddison (2002) show that the NCA can overlook stochastic processes and misidentify historical events. They suggest a

more statistical approach to phylogeography, but do not provide an alternative methodology.

1.3.3.2 DNA Regions Used in Phylogeography

The chloroplast DNA genome (cpDNA) has been the molecular marker of choice in phylogeographical studies of plants, because it is uniparentally (most often maternally) transmitted without intermolecular recombination and has various mutation rates within its different loci (Avise, 1998). One cpDNA region frequently used in higher level analyses is the non-coding *trn*L-F intergenic spacer, which although conservative in most angiosperms (Taberlet et al., 1991), is sufficiently polymorphic in bryophytes for population level studies (e.g. McDaniel and Shaw, 2003; Shaw et al., 2003).

The internal transcribed spacer (ITS) regions of nuclear rDNA can offer additional phylogeographical insight. Because nuclear and chloroplast DNA are under different evolutionary constraints in terms of inheritance and mutation rates, integrating both genomes can often reveal compelling phylogeographic patterns (Schaal et al., 1998). In the near future, as complications stemming from intermolecular recombination are resolved, nuclear phylogeography will result in more comprehensive population histories (Hare, 2001).

1.3.3.3 Phylogeography and Climate Change

Phylogeography is a tool with many uses. It has been used to reveal cryptic species (Shaw, 2000), deeply divergent lineages (Verheyen et al., 2003) and shared evolutionary histories (Arbogast and Kenagy, 2001).

As a sub-discipline of biogeography (Avise, 2000), phylogeography has a certain symbiotic relationship with palaeoenvironmental studies. Several phylogeographical studies have used available fossil data to corroborate theories on past species distributions and movements (e.g. Bermingham and Mortiz, 1998; Cottrell et al., 2002; Dumolin-Lapegue et al., 1997), while historical species ranges have served as proxy data sources for palaeoclimatic hypotheses (e.g. Meadows and Sugden, 1991).

The compilation of phylogeographic information of taxa from the same region, especially in the absence of a clear, continuous fossil record, can enhance the understanding of past environments and their evolutionary consequences on species (Cruzan and Templeton, 2000). One of the most exciting applications of phylogeographical analyses has been the reconstruction of post-glacial range expansions by several Northern hemisphere plant and animal taxa following the last ice age (e.g. Arbogast and Kenagy, 2001; Hewitt, 1999; Soltis et al., 1997)

1.3.3.4 Phylogeography Using Bryophytes

Bryophytes are particularly suitable study taxa for phylogeographical analyses. Many species have higher than expected levels of genetic diversity, low dispersal rates,

broad geographical ranges and evolutionary persistence. Their unique life cycle means that specimen material is haploid, which precludes the problematic multiple-band amplification of nuclear DNA often encountered in diploid plants.

In the past, bryophytes were assumed to be genetically poor organisms with low evolutionary potential. This theory was based on their haploid-dominant life cycle, high number of species with broad, disjunct distributions, high incidence of asexual reproduction, and short sperm dispersal distances (Dewey, 1989). The recent finding that mosses show inter-population genetic diversity greater than some angiosperms has reversed thinking and lead to intense speculation on the factors involved (Shaw, 2000).

Liverworts contain lower levels of genetic diversity than mosses, but higher levels than expected for haploid-dominant organisms (Wyatt, 1994). They tend to occur in small, isolated populations, with most diversity observed among rather than within populations, a pattern that may have resulted from repeated dispersal and founder events (Bischler and Boisselier-Dubayle, 1997) and that strengthens phylogeographic inference.

Even though asexual reproduction in bryophytes is very common and shown to be correlated with rarity in dioecious species (Laaka-Linberg et al., 2000), many species have intercontinental distributions (Schuster, 1966) that allow phylogeographic studies on global scales (e.g. McDaniel and Shaw, 2003). Furthermore, liverwort genetic diversity is generally found to be as high on a local scale than between inter-

continentally disjunct populations (Bischler and Boisselier-Dubayle, 1997), a significant advantage for regional studies.

Bryophytes have an evolutionary history long enough for phylogeographic reconstructions over extensive temporal scales. Many disjunctive bryophyte populations show partitioning similar to angiosperms, which may suggest ancient vicariant events, such as the break-up of the Gondwanan landmass 80 million years ago (Shaw, 2000). Extant bryophytes preserved in amber have been found to be several million years old (Frahm, 2000), implying that some species survived dramatic environmental changes, including the Quaternary glacial-interglacial cycles (During, 2000).

Perhaps most importantly, bryophytes, and liverworts in particular, are characterised by limited dispersal capabilities, due to life history traits such as short sperm dispersal distances (Wyatt, 1994), short-lived sporophytes and habitat specificity (Bischler and Boisselier-Dubayle, 1997). This makes liverworts especially informative in phylogeographic studies, because they often show the well-preserved genetic structure (Cruzan and Templeton, 2000) necessary for significant historical inference.

1.4 Study Organism

My project examines the phylogeographic patterns of *Jamesoniella colorata* (Lehm.)Schiffn., a leafy liverwort within the family Lophoziaceae (as defined by Grolle and Long, 2000). *J. colorata* has a disjunct, austral-subantarctic distribution and has been collected in South America, Tasmania, New Zealand, South Africa and

several subantarctic islands (Schuster, 2002). Figure 3 shows the global distribution of *J. colorata* and a typical gametophytic individual.

Within South Africa, *J. colorata* has been found in humid montane niches typically higher than 500 metres, where populations vary from one or two individuals to large mats containing many hundreds. It occurs from the mountain ranges of the Cape Peninsula east and north to KwaZulu-Natal (figure 4), where specimens have been collected in the Drakensberg at elevations as high as 3,000 metres (Arnell, 1963). This study focuses exclusively on populations that occur in the Western Cape Province, which falls within the climatic regions WRZ and YRZ, as discussed in section 1.2.

Life history characters play a major role in the genetic structure and distribution of *J. colorata*. As a dioecious species that rarely produces sporophytes, *J. colorata* probably relies heavily on asexual reproduction through clonal growth or dispersal of propagules (Gradstein et al., 2001). This may result in some populations becoming dominated by a few haplotypes, which augments among-population diversity (Wyatt, 1994). If sexual reproduction does occur in populations of *J. colorata*, the short-lived sporophyte allows a narrow window of opportunity for dispersal, although it has been shown that some liverwort spores are easily carried long distances by wind (Bischler and Boisselier-Dubayle, 1997).

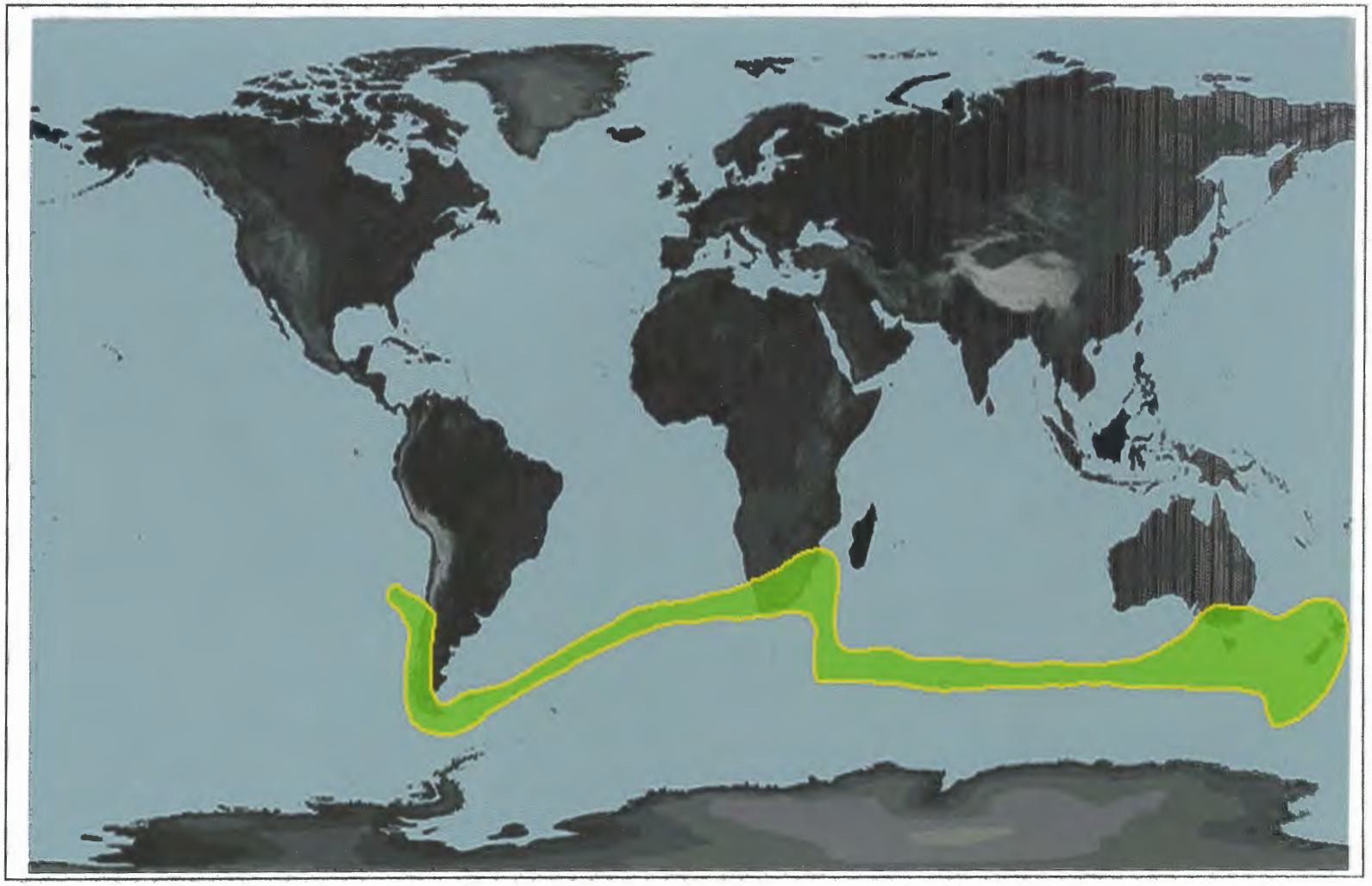

Figure 3. Global distribution of *Jamesoniella colorata* from Schuster (1966). Populations have been recorded in South America, South Africa, Australia, Tasmania, New Zealand and on many sub-Antarctic islands.

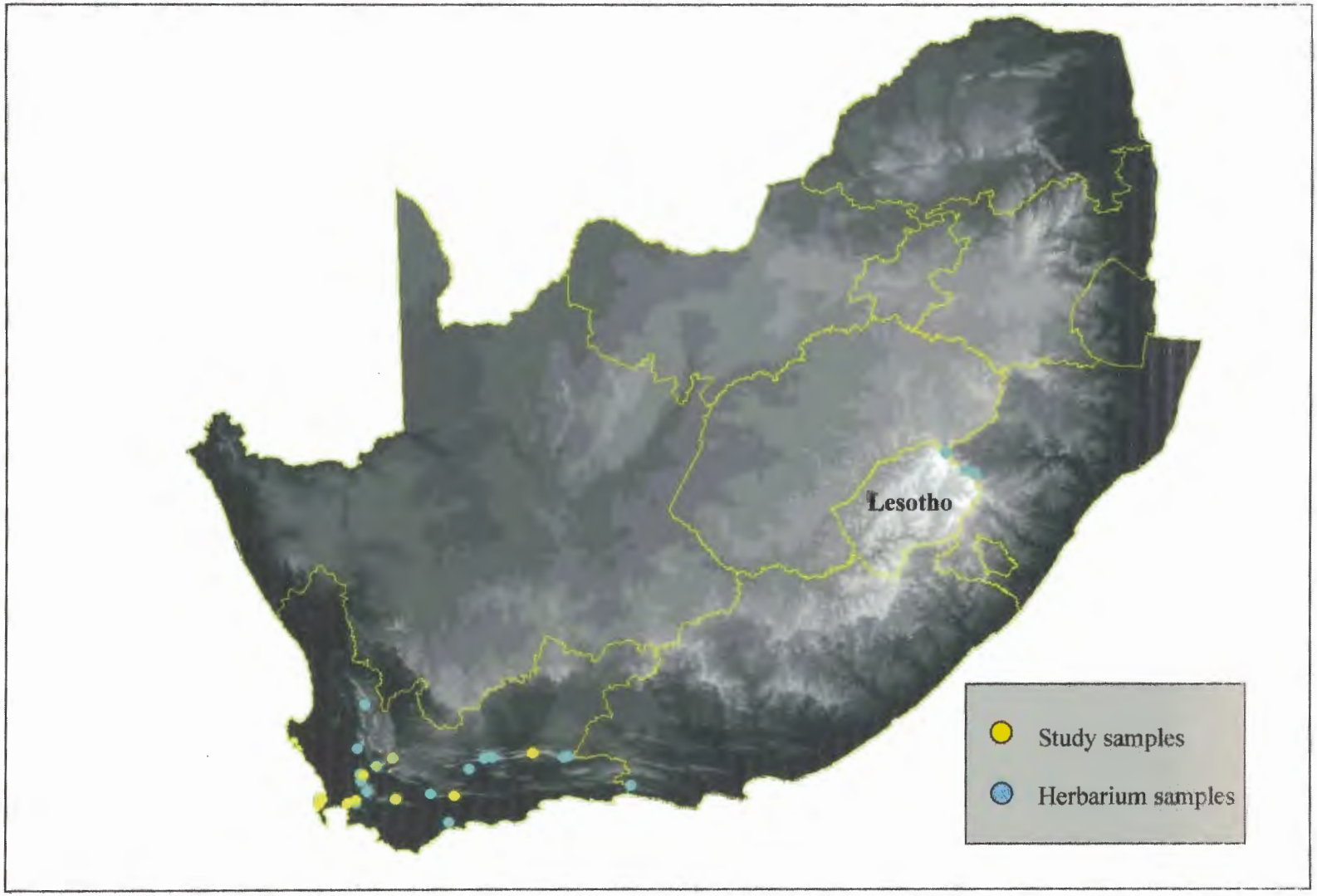

Figure 4. The entire known distribution of *J.colorata* in South Africa based on herbarium records and population sampling from this study. Outside of the study area (Western Cape province) the only other recorded collections occur in the Drakensburg Mountains of Lesotho.

1.5 Expected Genetic Patterns in *Jamesoniella colorata*

For montane species like *J. colorata*, the key mechanisms of genetic and demographic change during the Quaternary would have been population expansion into lower altitudes during cooler periods and range shrinkage into higher refugia during warming trends.

During cooler periods, such as the LGM, it is expected that *J. colorata* populations across the study area shifted to lower altitudes as suitable habitat increased. This range expansion is expected to have been characterised by an overall reduction in genetic diversity (Hewitt, 2000; however Widmer and Lexer, 2001), as colonisers dominated the gene pool of new populations (Hewitt, 1999).

In response to rainfall discrepancies, populations may have reacted differently along an east-west gradient. The drier LGM experienced by YRZ populations may have limited the expansion of *J. colorata*, while the wetter peaks of the WRZ may have offered populations greater expansion potential. However, substrate and other habitat preferences probably prevented *J. colorata* from becoming widely distributed, particularly in low altitudes.

Significant warming trends like the HA are expected to have led *J. colorata* to retreat to cooler, higher peaks in the WRZ and YRZ. In refugia, gene flow ceases as populations become geographically restricted, a common occurrence in liverworts due to their limited dispersal and specific habitat requirements (Bischler and Boisselier-Dubayle, 1997). Restricted populations of *J. colorata* may have been susceptible to

genetic drift and/or prolific asexual reproduction (Ellstrand and Elam, 1993), ultimately leading to higher genetic diversity among populations.

During a refugial phase, the steep and varied montane topography of the study area may have allowed populations of *J. colorata* to shift into niches with distinct microclimates, where lineages could persist and diverge. This possible mechanism was suggested by Midgley et al (2001), who noted that refugia in the Cape Fold Mountains during interglacial periods may have led to island-like isolation and the prolific diversification of species that characterises the Cape flora.

1.6 Aims and Objectives

This thesis is part of an international project on life history, biodiversity and rarity patterns of Lophoziaceae. It aims to provide a general understanding of levels of genetic variation and population history of species in this poorly understood family. In addition, this research may contribute to future studies on the climatic and evolutionary history of the CFR.

The main aims of this work are:

1) To define the regional extent and structure of genetic variation in populations of *J. colorata;*

2) To identify any significant demographic or historical events using population genetics and phylogeographical methods;

3) To decipher these events in light of possible regional palaeoclimatic trends; and,

4) To develop a bioclimatic envelope of *J. colorata* in order to better understand its present distribution in South Africa and also to infer the level of sampling error in this study.

Strategies used to achieve these aims are:

1) AMOVA analysis will be carried out on chloroplast and nuclear DNA sequence data to ascertain levels of genetic differentiation between and among populations of *J. colorata*;

2) Demographic processes will be identified from mismatch distributions and significant historical events will be inferred from NCA results;

3) The interpretation of *J. colorata* population history and its expected genetic patterns will be examined against the backdrop of palaeoclimatic trends in South Africa, and

4) A map of favourable habitat in South Africa will be constructed with Geographical Information Systems (GIS) software by projecting key environmental parameters correlated with the known distribution of *J.*

colorata. Results will be compared with its recorded and sampled distribution to identify potential sampling gaps.

2 Materials and Methods

2.1 Population Sampling and Storage

86 specimens of *J. colorata* were collected from 11 populations across the Western Cape Province, which accounts for the majority of its distribution in South Africa. Collection localities were selected by studying literature and specimen records housed in the Bolus Herbarium (BOL) at the University of Cape Town and by estimating sites based on the species' habitat preferences. Table 2 lists information for each sample population and figure 5 illustrates the position of each sampled population.

Table 2. Collection localities, number of individuals sampled, geographic co-ordinates, altitude and voucher reference numbers for all sampled populations.

	Population Locality and Abbreviation	No. of Samples	Geographic Co-Ordinates	Altitude (m)	Voucher Nos.
1	Horseshoe Ravine, Table Mtn. (HR)	12	33° 58' 30" S 18° 25' 40" E	600	RC 2-13
2	Nursery Ravine, Table Mtn. (NR)	13	33° 59' 00" S 18° 25' 00" E	760	RC 14-26
3	Constantia Berg (CB)	6	34° 03' 30" S 18° 23' 00" E	930	RC 27-32
4	Jonkershoek, Hottentots Holland Mtns (JH)	12	34° 00' 40" S 19° 00' 15" E	1200	RC 33-44
5	Helderberg (HB)	7	34° 02' 30" S 19° 52' 30" E	750	RC 45-51
6	Boesmanskloof, Riviersronderend Mtns. (BK)	10	34° 00' 00" S 19° 42' 00" E	700	TLN 212-220
7	Upper cable car station, Table Mtn. (CC)	8	33° 57' 30" S 18° 24' 00" E	1000	RC 62-68
8	Tradouw's Pass, Langeberg (TP)	2	33° 58' 30" S 20° 42' 30" E	340	RC 69-70
9	Bobbenjaansrivier, Limietberg (BJ)	3	33° 37' 30" S 19° 09' 00" E	1000	RC 71-73
10	Swartberg Pass, Groot Swartberg (SB)	8	33° 21' 30" S 22° 03' 00" E	1600	RC 74-81
11	Matroosberg, Hex River Mtns. (MB)	5	33° 23' 00" S 19° 40' 30" E	2250	RC 81-86

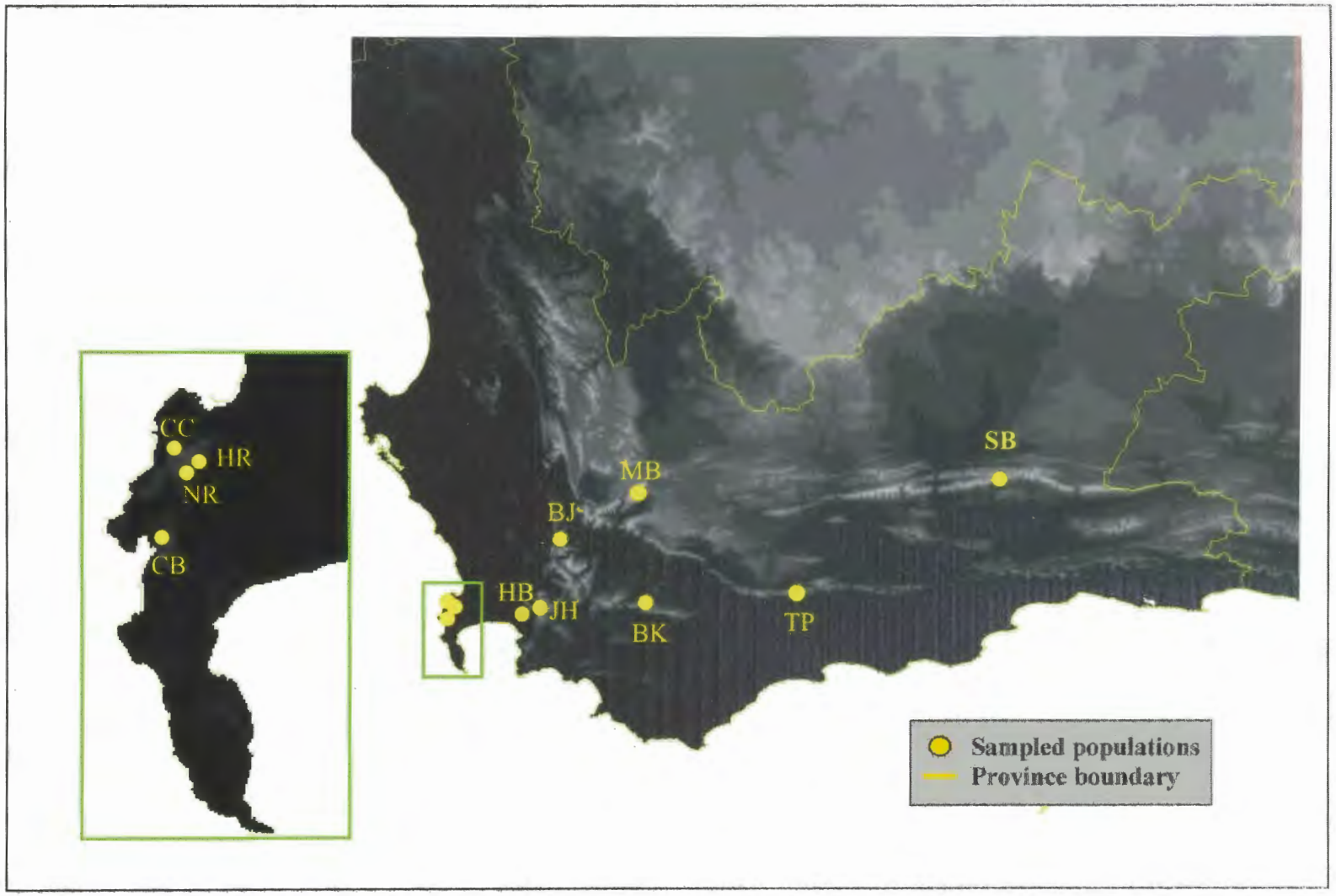

Figure 5. Distribution of the 11 populations of *J.colorata* sampled in the Western Cape province for this study, with inset on the Cape Peninsula. Additional information on populations, including the abbreviations used here, is found in table 2.

In order to attain meaningful phylogeographic estimations, efforts were made to sample across the entire study area and to collect at least ten individuals from each population. However, small population sizes and difficult montane terrain limited the average sample size to eight individuals.

Each specimen consisted exclusively of gametophytic material weighing approximately 3 grams. Specimens were dried and stored in paper envelopes labelled with collection numbers and locality details. All voucher specimens were deposited in BOL.

2.2 DNA Extraction, Amplification and Sequencing

Material for extraction was selected by carefully pruning approximately 10 milligrams of healthy, clean shoots with leaves under a dissecting microscope, which were placed in 1.5 millilitre micro-centrifuge tubes.

DNA was extracted following a modified version of the Doyle and Doyle (1987) protocol. Each sample of dried material was ground in liquid nitrogen and then incubated at 65°C for approximately an hour in 700 µl of preheated hexadectyltrimethylammonium bromide (CTAB) and _-mercaptoethanol. 600 µl of chloroform-isoamyl alcohol (24:1 v/v) was added and mixed by inversion. Samples were spun for five minutes and the aqueous phase was transferred into a clean micro-centrifuge tube. An equal volume of ice-cold isopropanol was added and tubes were stored in a -10°C degree freezer overnight to precipitate the DNA. The samples were

spun and washed with 75% ethanol. The samples were left to dry overnight and then resuspended with 50 µl of TE (10 mM Tris-Cl pH 7.4, 1 mM EDTA pH 8.0).

Primers *trn*C and *trn*F (Taberlet et al., 1991) were used to isolate the chloroplast *trn*L-F region while the nuclear ITS 1 region was amplified with 18KRC and ITS2 primers (Hamby, 1988; White et al., 1990). Primer sequences are shown in table 3. Target DNA regions were amplified by Polymerase Chain Reaction (PCR) using 0.75 units of BIOTAQ™ DNA polymerase (Bioline) in 30 µl volumes also containing 1 X NH_4 buffer and 5mM $MgCl_2$, 0.1 mM of each dNTP and 0.3µM of each primer, with 3µl of unquantified diluted DNA template. Thermo-cycling was carried out on a Hybaid Sprint set to the following thermal conditions: initial denaturation at 97° C for two mins, followed by 30 cycles of 97° C for 1 min, 52° C for one min, 72° C for two mins and a final polymerisation step at 72° C for seven mins.

Table 3. Primers used in direct DNA sequencing.

Genome	Region And Direction	Primer	Sequence (5'- 3')	Reference
Chloroplast	*Trn*L-F			
	forward	*trn* C	CGAAATCGGTAGACGCTACG	Taberlet et al. 1991
	reverse	*trn* F	TTTGAACTGGTGACACGAG	Taberlet et al. 1991
Nuclear	ITS			
	forward	18KRC	GCACGCGCGCTACACTGA	Hamby et al. 1988
	reverse	ITS2	GCTGCGTTCTTCATCGATGC	White et al. 1990

The amplified DNA was cleaned using GFX™ PCR DNA and Gel Band Purification Kits (Amersham Biosciences). Cycle sequencing was carried out by PCR in 10µl volumes containing the following: 2 µl of BigDye® Terminator v1.1 5 X Sequencing

Buffer (Applied Biosystems), 1 μl of BigDye® Terminator v3.1 Cycle Sequencing RR-100 (Applied Biosystems), 0.16 μl primer, 1 to 4 μl DNA template, and the remaining volume of nanopure water. Cycle sequencing products were resolved on an ABI PRISM 3100 Genetic Analyzer for direct nucleotide sequencing.

2.3 Phylogenetic and Phylogeographical Analyses

Chloroplast and nuclear data were analysed separately. Sequences were assembled on SeqMan (LaserGene System Software, DNAStar, Inc.) and aligned manually using MegAlign (LaserGene System Software, DNAStar, Inc.). Alignment ends were trimmed to exclude missing data and indels were coded as present or absent.

Haplotype distributions, AMOVA statistics and pairwise differences between haplotypes were calculated using Arlequin version 2.0 (Schneider et al., 2000). Mismatch distribution histograms were made in Microsoft® Excel 2000.

Sequence data were analysed using the nested clade approach following Templeton and Sing (1993). TCS version 1.13 (Clement et al., 2000) was used to construct a statistical parsimony network that was manually arranged into a nested cladogram. GeoDis version 2.0 (Posada et al., 2000) was used to estimate clade and nested clade distances, which were then interpreted with the Inference Key developed by Templeton et al. (1995) to determine the most likely historical explanations of significant distance values.

2.4 Bio-climatic Envelope

A map showing the distribution of favourable habitat in the Western Cape was constructed using Geographical Information Systems (GIS) software (ArcView version 3.2, Environmental Systems Research Institute, Inc.). The process involved three principal steps.

First, decimal degrees geographical co-ordinates of the 39 populations of *J. colorata* recorded from BOL and this project were compiled. Next, the locations were projected onto a map of South Africa containing the baseline parameters of rainfall, maximum temperature, minimum temperature, elevation, aspect and slope, as sourced from the South African Atlas of Agrohydrology and Agroclimatology (Schulze, 1997). This step resulted in a suite of bioclimatic correlates that were statistically transformed into mean, maximum and minimum values. Lastly, the baseline data map was queried for the parameter correlate maximum and minimum ranges, to produce a distribution layer of favourable habitat consisting of areas that fall into the ranges of *all* correlate ranges.

3 Results

3.1 Observed Distribution and Habitat

The recorded distribution of *J. colorata* in South Africa, based primarily on herbarium records, is restricted to high altitudes of major mountain ranges. Field observations from this study confirm that these peaks receive considerable moisture, and that the wettest areas, such as Table Mountain and the Hottentots Holland Mountains, have the most abundant populations. The 20 km^2 of Table Mountain alone is home to over 11 recorded populations of *J. colorata.*

Adequate moisture and rocky substrate appear to be the primary factors behind its high altitude habitat preference. Moving inland from the coastal populations, abundance within populations decreases and distance between populations increases. In the drier environments of the inland ranges, is appears that populations compensate by seeking out the increased moisture and reduced evapo-transpiration of the highest elevations. This can be illustrated by comparing population data in table 2. As an example, the highest population, MB, (2250 m, 5 samples) was smaller than the lower JH population (1600 m, 12 samples), which being closer to the coast, receives more annual rainfall.

3.2 Palaeoclimatic Divisions

All sampled populations occur within the WRZ except the BK, MB, TP and SB populations, which lie within the YRZ. The BK population is found just east of the WRZ and YRZ boundary. However, because climatic conditions change along a

gradient and are tempered along the coast, it may have experienced a climatic history more similar to the WRZ populations. No sampled populations occur in the SRZ.

3.3 *Trn*L-F Chloroplast Region

3.3.1 DNA Sequencing

58 samples were successfully sequenced (out of 86 samples collected) for the *trn*L-F cpDNA dataset. None of the herbarium specimens of *J. colorata* available from BOL were sequenced, as all were over fifty years old. The alignment was 496 nucleotide base pairs in length and contained 15 polymorphic sites and three insertion/deletions (indels). The alignment files can be found on the attached CD.

3.3.2 Phylogenetic Reconstruction

Four additional samples showed marked molecular and morphological dissimilarity from the rest of the dataset and are hypothesised to be *J. oenops*, another austral species that has been synonymised with *J. colorata* (Grolle, 1971). In order to determine their phylogenetic position, a midpoint-rooted Neighbour Joining (NJ) tree containing all samples was constructed with PAUP 4.0b10 (Swofford, 1998). The NJ tree is illustrated in figure 6, with the seven *trn*L-F haplotype clades labelled. The clade containing the four putative '*J. oenops*' sequences is sister to the rest of the tree and is subdivided into two branches with a Similarity Index of 96.6%. One '*J. oenops*' haplotype consists of two BK population samples, and the other comprises samples from Table Mountain (CC6 and NR11).

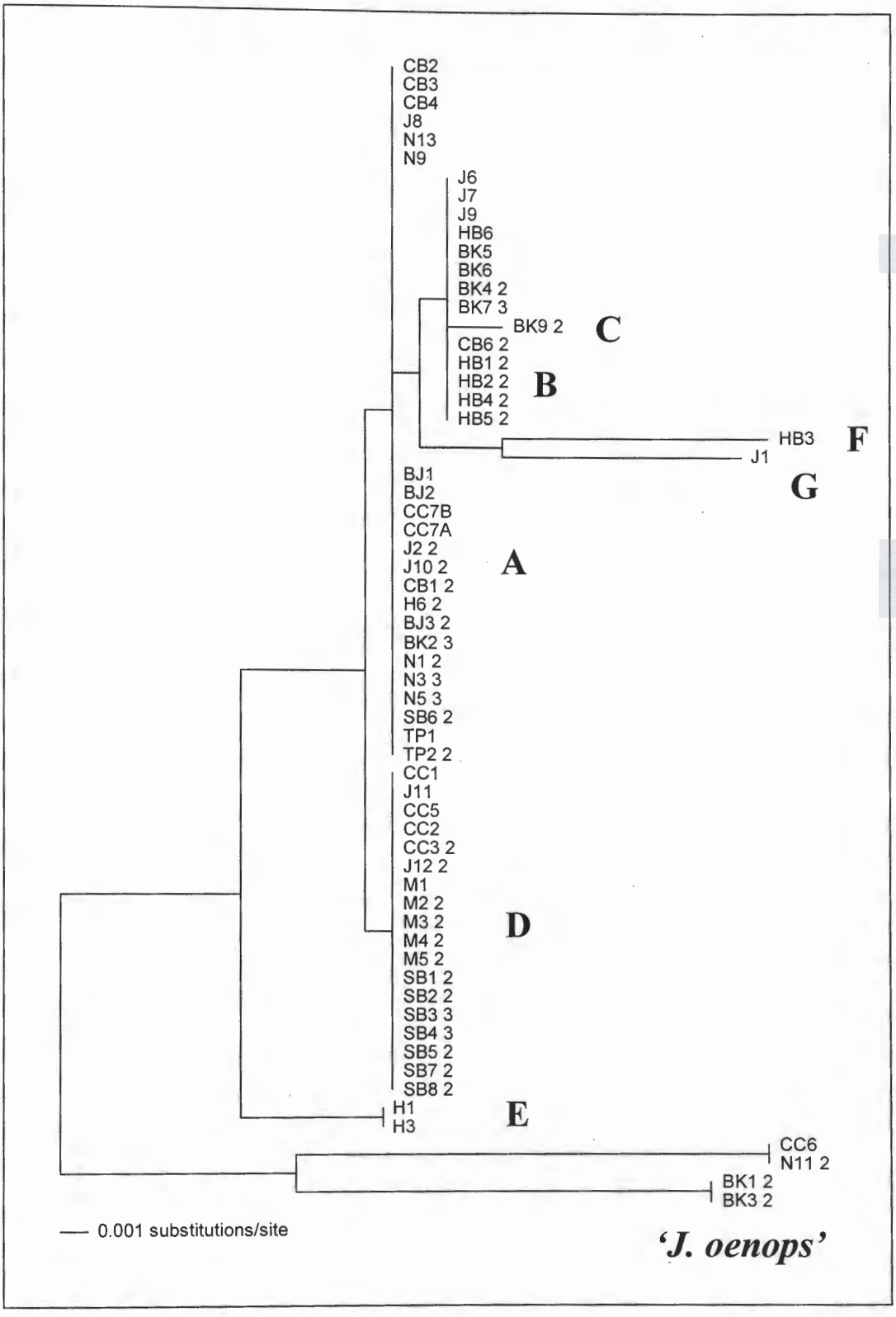

Figure 6. The midpoint-rooted Neighbour Joining tree for the *trn*L-F dataset, in which the 4 putative *J. oenops* samples are sister to the rest of the tree. Haplotypes groups of *J. colorata* are labelled to the right of the clades.

3.3.3 Population Structure

AMOVA analysis of the *trn*L-F dataset revealed that genetic variation is divided 54.6% within populations and 45.4% among populations. Seven different haplotypes were identified and their distribution among populations is shown in table 4.

Table 4. The distribution of seven haplotypes (A-G) among the 11 sampled populations of *J. colorata*.

Populations	**Haploytpes**							**Total Sequences per Population**
	A	**B**	**C**	**D**	**E**	**F**	**G**	
BJ	3							3
BK	1	4	1					6
CB	4	1						5
CC	2			4				6
HR	1				2			3
HB		5				1		6
JH	3	3		2			1	9
MB				5				5
NR	5							5
SB	1			7				8
TP	2							2
Total Samples per Haplotype	22	13	1	18	2	1	1	

Figure 7 depicts the haplotype frequencies, geographic distribution and positions in the *trn*L-F nested cladogram. Haplotype A is the most frequent and widespread, found in all populations except for MB and HB. The second most common haplotype D shows the most displacement, occurring in high frequencies among the outer populations, SB, MB and CC. Haplotype B has the third highest frequency, but is observed only in southwestern Cape populations. Among the tip haplotypes, C, F and G are restricted to the central area in populations BK, HB and JH, respectively. Haplotype E is unique to the CC population on Table Mountain.

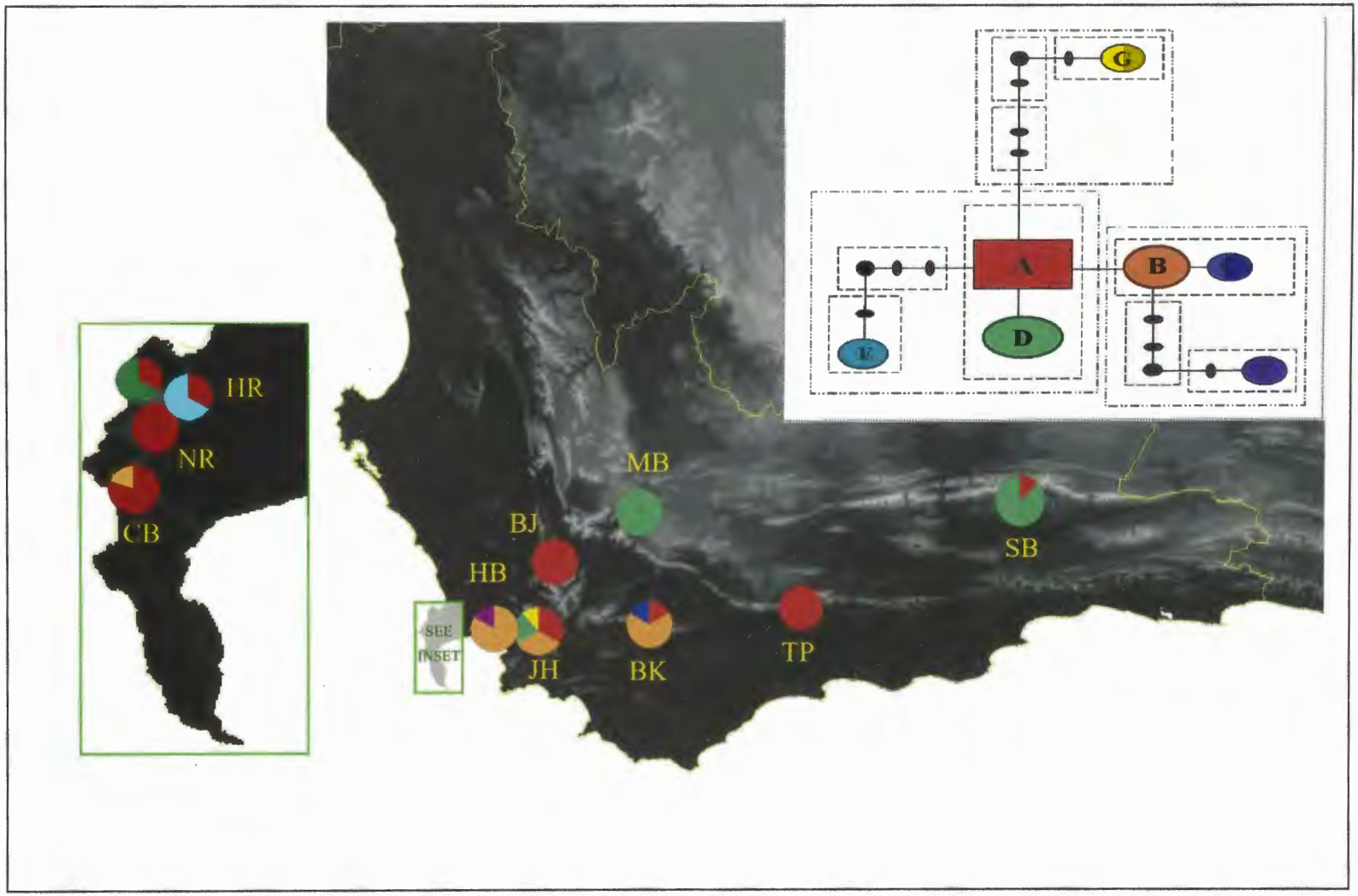

Figure 7. An illustration of the *trn*L-F haplotype frequencies for sampled populations of *J. colorata.* Colours correspond to haplotypes in the nested cladogram shown in the upper right window. The inset shows the relatively close Cape Peninsula populations.

3.3.4 Mismatch Distributions

Figure 8 shows the observed and simulated distribution of *trn*L-F pairwise differences in all individuals of *J. colorata*. The x-axis describes the number of nucleotide sites where polymorphisms occur and the y-axis tracks the number of pairwise comparisons with differences at those sites. The simulated line is based on a coalescent algorithm modified from Hudson (1990) and confidence percentile values (p) were calculated using a parametric bootstrap approach (Schneider et al., 2000).

The bimodal mismatch distribution indicates that *J. colorata* has undergone two population expansion events in the Western Cape Province: the more recent peak at x=1 and the older peak at x=6.

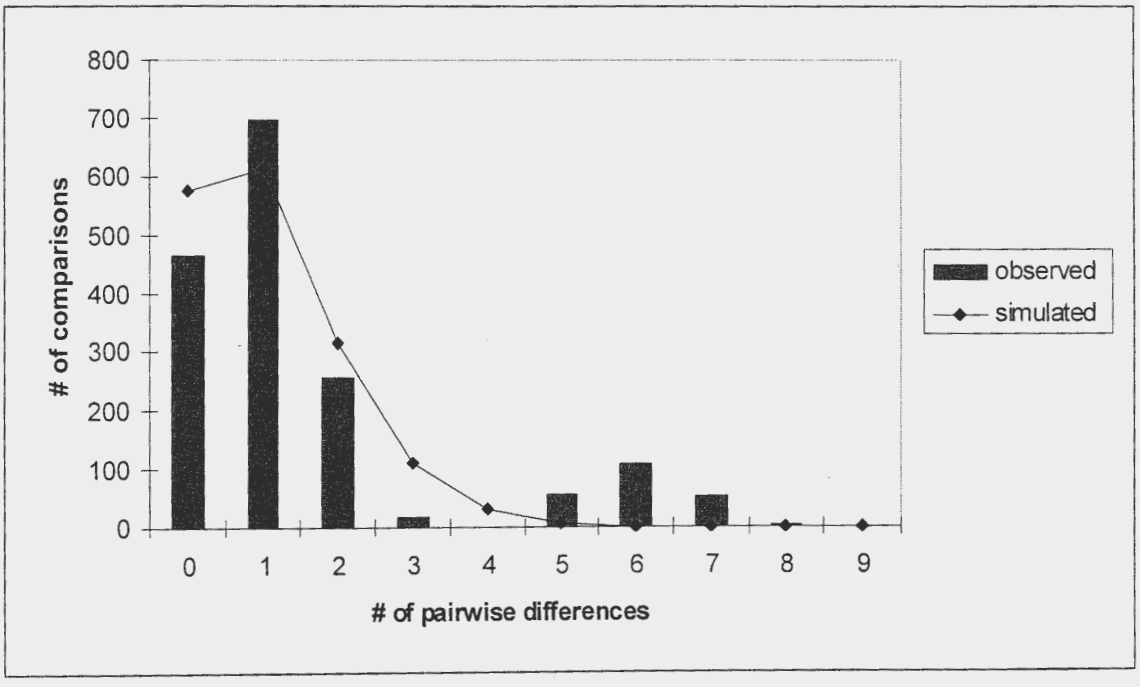

Figure 8. Mismatch distribution of pairwise nucleotide differences in the *trn*L-F dataset, showing two peaks of population expansion at x=1 and x=6. The confidence

percentile interval for the observed and simulated distributions is significant at p=0.018.

3.3.5 Haplotype Network

The nested cladogram of the seven *trn*L-F haplotypes is shown in detail in figure 9. It is highly structured, with nine level-one clades and three level-two clades. The most frequent and widespread haploytype (A) roots the cladogram. Haplotypes E, F and G show the most divergence from their origin, each preceded by at least five mutational steps.

3.3.6 Nested Clade Analysis

Table 5 lists the clades in which the null hypothesis of 'no geographical correlation of genetic diversity' was rejected in the NCA. Clade 1-1 showed the most significant D_C and D_N values of the entire cladogram, which influenced the higher-level clade assessments. For example, the null hypothesis was rejected for clade 2-1 because of its significantly large Interior-Tip Dc value, which measures the difference of the interior clade (1-1) D_C from the tip clades D_C (1-2 and 1-6). Significant haplotype distances were also detected in the overall network (clade 3-1) in which the Restricted Gene Flow inference is strengthened by the diverse locations of the sub-clades with restricted distributions (e.g. clades (and populations) 1-2 (HR), 1-5 (JH), and 2-2 (HB)).

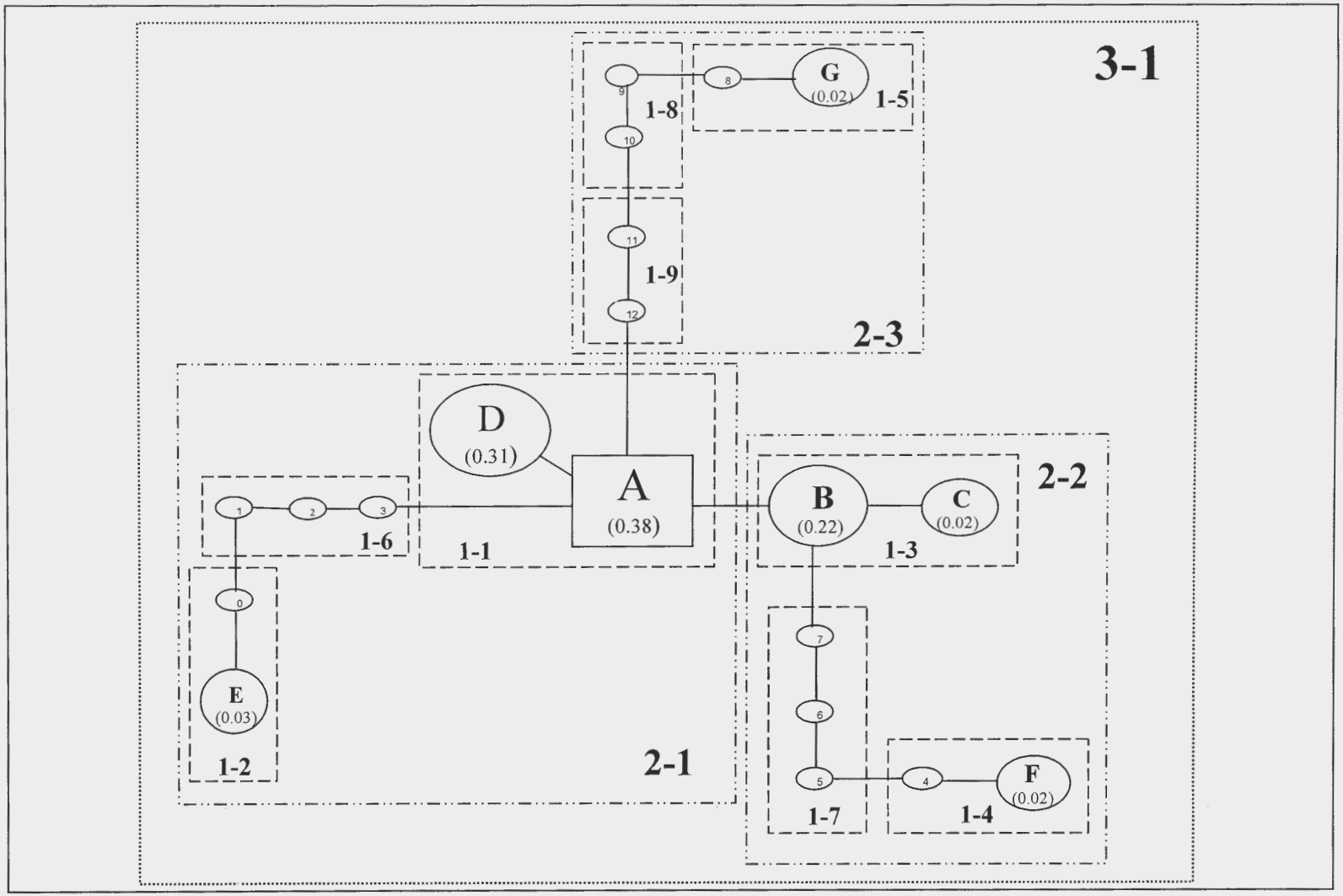

Figure 9. The nested haplotype network used in the NCA for the *trn*L-F dataset. Relative haplotype frequencies are shown under each letter. Smaller numbered circles represent missing haplotypes that are assumed to be unsampled or extinct.

Table 5. NCA results for *trn*L-F dataset. Haplotypes and clades are illustrated in figure 9 and population abbreviations are from table 2. Inference Path refers to steps taken in the dichotomous inference key (Templeton, 1998).

Clade	Haplotypes	Populations	Inference Path And Conclusion
1-1	A, D	All except HB	1/2/11/12 - Continuous Range Expansion
2-1	A, D, E	All except HB	1/2/3/4 - Isolation by Restricted Gene Flow
3-1	All	All	1/2/3/4 - Isolation by Restricted Gene Flow

3.4 ITS Nuclear Region

3.4.1 DNA Sequencing

The amplification of the nuclear ITS region was problematic and resulted in only 14 sequences from six populations. Sequences from the NR, HR and CC populations were grouped into the Table Mountain (TM) population to facilitate analyses. The ITS alignment contained 11 polymorphic sites and no indels out of 686 nucleotide base pairs. The alignment files can be found on the attached CD.

The nuclear primers used (18KRC and ITS2) are applicable for a wide range of taxa, and as a result successfully amplified fungal elements that are known to associate with leafy liverworts (Read et al., 2000), resulting in incompatible or partial sequences. GenBank's nucleotide-nucleotide BLAST search (National Centre for Biotechnology Information website) revealed that the questionable sequences were most similar to species from phylum Ascomycota.

3.4.2 Population Structure

Due to the low sampling size of the ITS sequence dataset, the AMOVA results indicate no significant genetic structuring among populations. The distribution of five haplotypes among the three populations is presented in table 6.

Table 6. The distribution of the five ITS haplotypes among three populations.

Populations	**Haploytpes**					**Total Samples per Population**
	A	**B**	**C**	**D**	**E**	
JH	4	2		1	1	8
CB	2					2
TM	2	1	1			4
Total Samples per Haplotype	8	3	1	1	1	

Figure 10 shows the haplotype frequencies, geographic distribution and positions in the ITS nested cladogram. Haplotype A makes up at least half of the diversity in all three populations. The second most frequent haplotype B is found in a quarter of the samples from the JH and TM populations. Haplotype C is highly derived and restricted to the CC population, while haplotypes D and E, both closely related to haplotype A, are only found in the JH population.

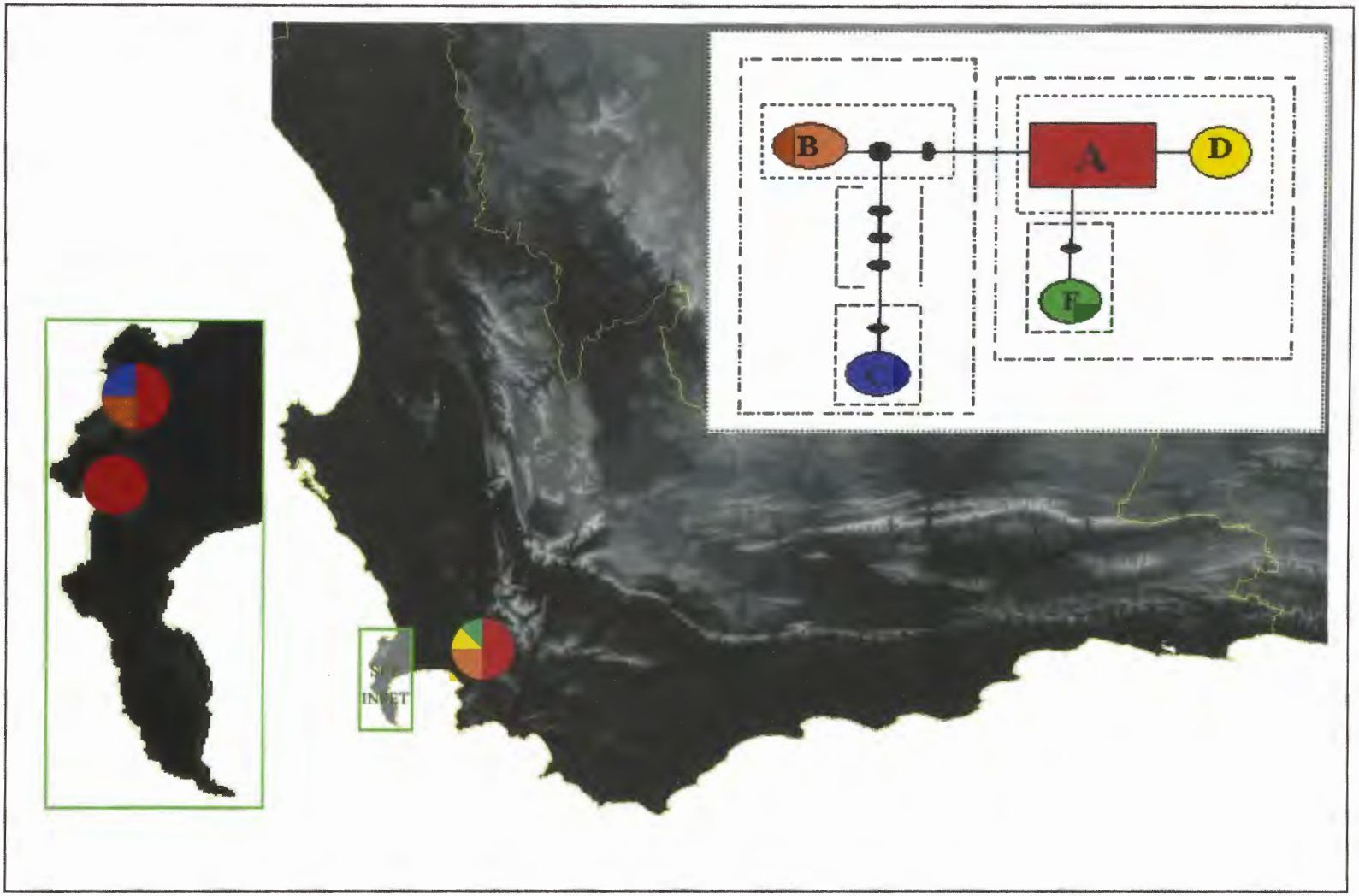

Figure 10. An illustration of the ITS haplotype frequencies for sampled populations of *J. colorata.* Colours correspond to haplotypes in the nested cladogram shown in the upper right window. The inset shows the relatively close Cape Peninsula populations..

3.4.3 *Mismatch Distribution*

Figure 11 depicts the observed and simulated mismatch distribution for the ITS dataset (see 3.3.4 for description of graph and methods). As in the *trn*L-F mismatch distribution, two peaks are clearly evident on the graph, indicating a recent (x=0) and an older population expansion event (x=3).

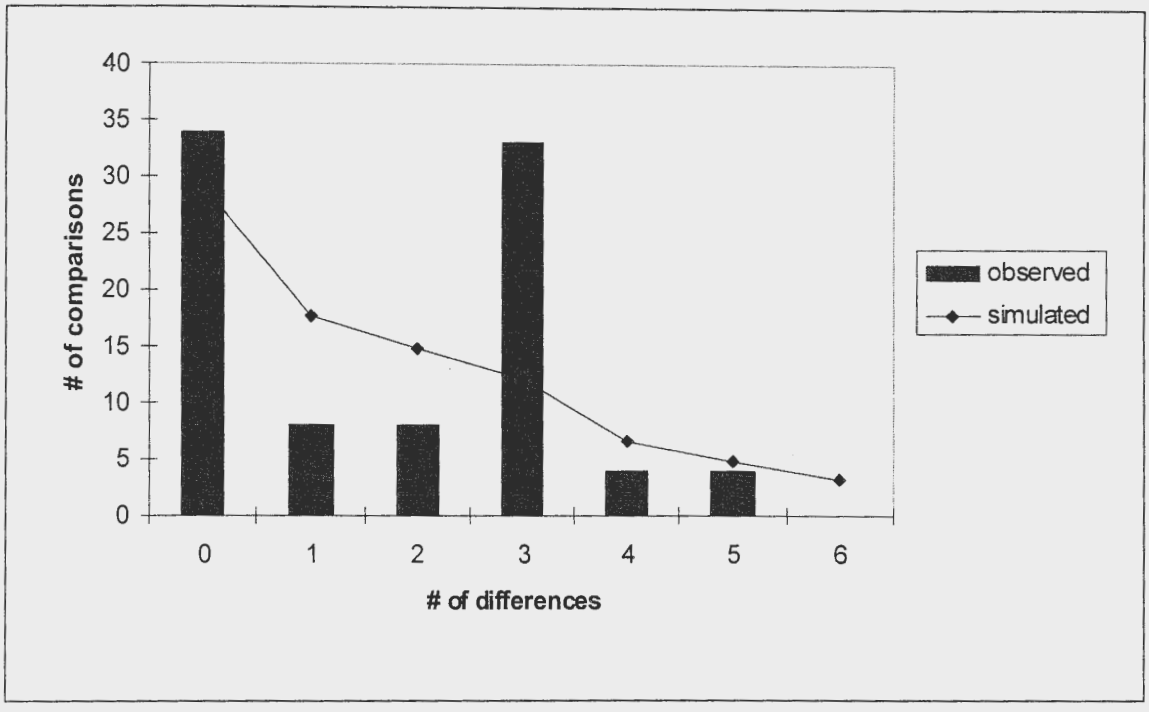

Figure 11. The mismatch distribution for the ITS sequence dataset. A bimodal trend is evident, indicating expansion events at x=0 and x=3. The confidence percentile interval is not significant at p=0.13.

3.4.4 *Haplotype Network*

Figure 12 shows the ITS haplotype network used for the NCA. It contains five level-one clades and two level-two clades. Haplotype A has the highest frequency and is the

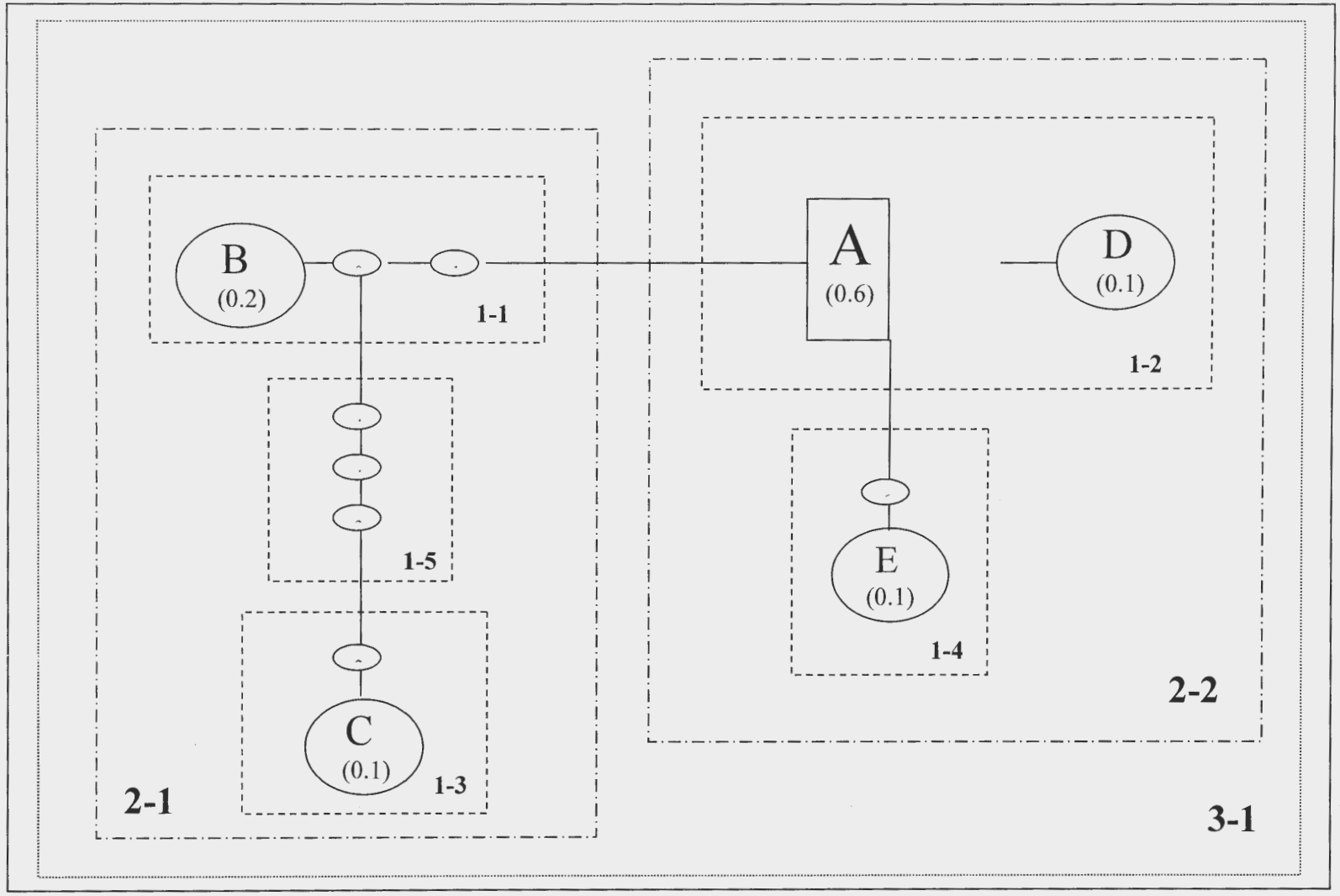

Figure 12. The nested haplotype network used in the NCA for the ITS dataset. Relative haplotype frequencies are shown under each letter. Smaller numbered circles represent missing haplotypes that are assumed to be unsampled or extinct.

ancestral root of the nested cladogram. The most diverged haplotype is C, which lies seven mutational steps from the ancestral haplotype.

3.4.5 Nested Clade Analysis

All NCA distance values for ITS were insignificant ($p>0.05$) and therefore no historical factors were inferred. This is certainly the result of the small size and narrow geographic range of the sequenced samples, which ultimately precluded any meaningful comparisons of D_C and D_N values.

3.5 Bioclimatic Envelope

Table 7 lists the environmental parameter values used to construct the GIS bioclimatic envelope for *J. colorata*, which is shown in figure 13.

The applicability of each parameter correlate for the exercise was assessed individually, and aspect and elevation were determined to be unsuitable. Aspect was eliminated because the range of values was too wide, and therefore would not be useful in specifying favourable habitat. The elevation correlate was regarded as a product of other habitat constraints on *J. colorata*, such as temperature, rainfall and possibly edaphic conditions (i.e. rocky substrate). Based on extensive field observations and known species parameters, rainfall, temperature and slope were considered to be the most influential factors in the distribution of *J. colorata.*

Table 7. Statistical values of environmental parameter correlates, with shading indicating the values used to construct the bioclimatic envelope for *J. colorata*.

Parameters	Mean	Maximum	Minimum
Rainfall (mm)	82.0	203	21.5
Temp Maximum (C°)	18.8	23.8	12.7
Temp Minimum (C°)	7.94	11.6	0.74
Slope (degrees)	20.4	48.1	2.36
Aspect (degrees)	179	357	25.6
Elevation (m)	1104	2994	373

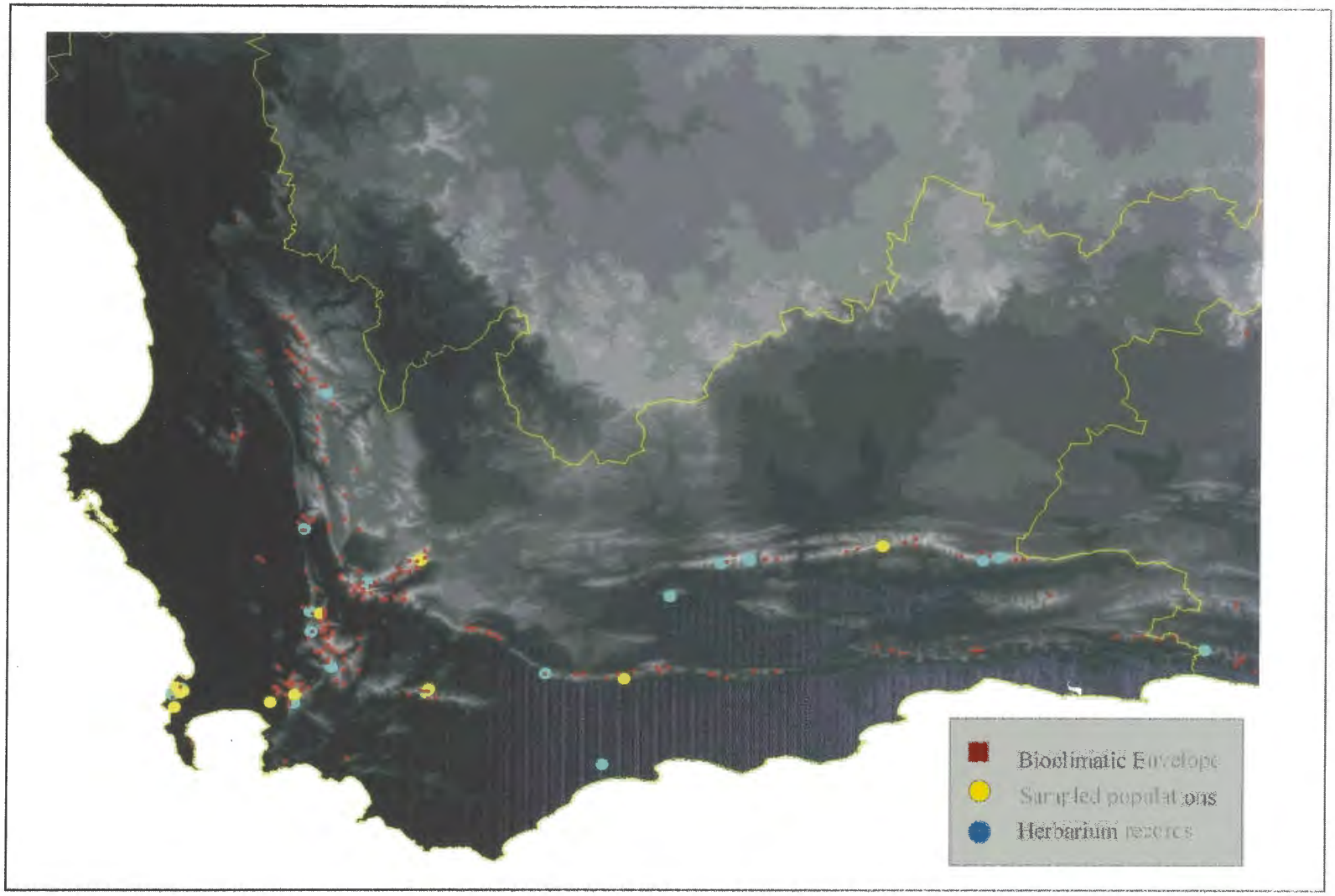

Figure 13. GIS Bioclimatic Envelope showing favourable habitat (in red) and all recorded and sampled populations of *J. colorata* in the Western Cape Province.

4.0 Discussion

4.1 Bioclimatic Envelope

The bioclimatic envelope broadly matches the recorded and observed habitat of *J. colorata* in South Africa and indicates that sampling in this study covered the distribution relatively well. The envelope shows that most of the favourable habitat occurs in the southwest peaks of the Cape Fold Mountains, with sparsely scattered pockets extending north to the Cederberg and east as far as the Tsitstikamma mountains. Although not shown in figure 13, a small patch of favourable habitat also exists outside the study area in the Drakensberg, which corresponds well with herbarium records.

Several areas identified from the envelope have not been sampled in this study. The Cederberg, Groot Winterhoek, Langeberg and Tsitsikamma mountains are areas that should be targeted for future collecting in order to adequately cover gaps in sampling.

4.2 Significant Historical Factors

4.2.1 Restricted Gene Flow

The *trn*L-F NCA resulted in an inference of Restricted Gene Flow by Limited Dispersal for the outermost nested clade (clade 3-1), which encompasses all populations. Under the associated model provided by Templeton (1998), most mutational derivatives of the ancestral haplotype are expected to occur near their origin, which in this case lies in the southwestern Cape, centred around the Hottentots

Holland Mountains. In this area, populations JH and BK have the highest haplotype diversity, and four unique haplotypes occur in populations JH, BK, HB and CC.

Also expected in the restricted gene flow model is a strong tendency for interior haplotypes to be widespread (A, B and D) while tip haplotypes occur in smaller ranges. This is seen in haplotypes C, E, F and G, which are restricted to populations BK, HR, HB and JH, respectively.

Clade 2-1 also had significant values that led to a Restricted Gene Flow inference. This was driven by the more widespread and distant (from the geographical clade centre) haplotypes A and D in the interior clade (1-1), as compared to tip haplotype E, which is unique to population HR.

The emerging picture thus far is one of a core group of older, more frequent and widespread haplotypes (A, B, and D) and a suite of descendant haplotypes (C, E, F and G) that have experienced very little or no genetic exchange.

4.2.2 Range Expansion

The innermost nested clade (1-1) was significant for the Contiguous Range Expansion inference, because haplotype D is more displaced and dispersed throughout the study area than ancestral haplotype A. The effects of this expansion are apparent in population MB, where haplotype D is fixed, and in the CC and SB populations, where it enjoys high frequencies.

4.3 Inferred History of *J. colorata*

In this section, the combined results of the AMOVA, mismatch distributions and NCA are used to develop a hypothetical history of *J. colorata* in the Western Cape Province. The underlying caveat in this reconstruction, and indeed in all phylogeographical studies, is that sampling gaps may contain crucial information that could alter this proposed scenario.

Initially, ancestral haplotype A dominated populations that were concentrated in the southwestern Cape (JH, BK and Cape Peninsula populations). One-step mutations resulted in haplotypes B and D, which, although not as common as A, increased in frequency in populations near the ancestral origin. This increase is reflected by the older expansion event present in the mismatch distributions of both DNA regions.

At some point, mutations near the origin increased as ancestral populations became smaller and experienced the effects of bottlenecks and genetic drift. This is supported by the high among-population diversity detected in the AMOVA (Fst = 0.45), and suggests a period of genetic diversification in upper montane refugia. Some of these mutations are apparent in contemporary populations (haplotypes C, E, F and G), but as seen in the *trn*L-F cladogram, even more were unsampled or became extinct.

Although the Cape Peninsula populations (CC, HR, NR and CB) are not as diverse as the other southwestern Cape populations, they contain ancestral haplotypes in varying frequencies and show a pattern of refugial divergence, primarily supported by the

unique haplotype E in the HR population. This high diversity is particularly notable given that the island-like isolation and relatively small area of Table Mountain.

Subsequently, with the arrival of cooler temperatures, haplotype D expanded northward and eastward into the Hex River Mountains and Swartberg, where it became fixed for two populations (MB and SB). This cooling trend and expansion event was probably relatively recent, as evidenced by the absence of mutational divergence in the colonised populations.

The eastward expansion hypothesis is further supported by the observation that the populations in the inland peaks are currently at the highest altitudes possible, which suggests that during warmer periods these populations would have become rare or extinct, facilitating colonisation by new haplotypes.

Haplotype A is also fixed for several populations in the inland mountain ranges, and as the ancestral and most common haplotype, probably expanded with haplotype D. However, the small sample sizes of the TP and BJ populations (two and three samples) prevent any realistic reconstruction of the recent history of haplotype A.

Nonetheless, the expansion of haplotype D is strongly supported by the NCA and most likely corresponds to the more recent peak of pairwise differences in the mismatch distributions.

4.4 Correlation with the Palaeoclimatic Record

The hypothetical history of *J. colorata* can be tested against the major climatic periods outlined in the palaeoclimatic review (section 1.2). The two historical population events examined in this section are the refugial phase that accompanied a warming period, and the ensuing range expansion brought on by a cooling trend.

4.4.1 Warming and Refugia

The Last Interglacial period, which peaked approximately 125,000 years BP and spanned 5,000-6,000 years, would have forced *J. colorata* into refugia. Regional rainfall patterns are not known for the Last Interglacial, but by comparing evidence from the LGM and present interglacial, it can be imagined that conditions were drier in the WRZ as compared to the surrounding glacial periods.

Another possible refugial period is the present interglacial, and in particular the HA, which occurred from ~8,000-4,000 years BP. Conditions at the HA are inferred as ~2°C warmer then present and drier in both the WRZ and YRZ. Given that the duration and conditions of these warming periods were similar, it is thought that *both* the last interglacial and the HA may have led to range contractions in populations of *J. colorata*.

The Medieval Warm Epoch (900-1300 AD) may have driven *J. colorata* populations into refugia. However, it was probably too brief and too recent to have significantly affected the observed levels of diversity of the slow-evolving *trn*L-F region. Further

work on ITS or other rapidly evolving DNA regions may reveal genetic impacts of this period.

4.4.2 Cooling and Range Expansion

There are two primary cooling periods that may have been associated with the range expansion of *J.colorata* populations inferred from the NCA and mismatch distributions. These are the last glacial period, between ~70,000 and 18,000 BP, and the post-HA cooling trend of the last ~4,000 years.

During the significant cooling of the last glacial period, populations of *J. colorata* would have had ample time to expand widely into suitable habitat. Both the WRZ and YRZ probably witnessed expansion events as a result of reduced evaporation and increased winter rainfall, although the exact boundaries of the WRZ at that time are unclear.

The record of late-Holocene cooling trend does not provide definite trends within the WRZ and YRZ because of the generally stable conditions of the Holocene, and the numerous low amplitude climatic fluctuations that have occurred over the last several millennia. However, both regions appear overall to have been slightly cooler and wetter compared to the HA (Meadows and Baxter, 1999; Scholtz, 1986).

Range expansion of *J. colorata* was possible during the late Holocene, but almost certainly to a lesser extent than during the last glacial period, due to the warmer temperatures. Also, post-HA rainfall increases in the WRZ are of a distinctly lower magnitude than those experienced in the last glacial period.

4.4.3 Expansion in the Southwestern Cape

If range expansion of *J. colorata* did occur in late Quaternary cooling periods, several factors would have favoured southwestern Cape populations as sources of expansion. In the Hottentots Holland and Cape Peninsula mountains, the summer southeast trade winds generate frequent cloud cover, which supplies sufficient moisture to support numerous montane plant species (Deacon et al., 1992). If this moisture continually mitigated the population mortality of *J. colorata* during hot, dry summers, populations would have had greater potential to expand to new habitats when conditions became ideal.

During the winter, the wet conditions that favour sexual reproduction and spore production in populations of *J. colorata* would have coincided with strong westerlies in the southwestern Cape. The combination of these events may have facilitated the eastward dispersal of spores and asexual propagules to colonise new habitats in the eastern part of the study area, as indicated in the *trn*L-F haplotype distribution.

4.4.4 Timing of Expansion Events

In principle, DNA region mutation rates can be used to assign dates to expansion episodes from mismatch distributions. These rates are usually calculated by extrapolating molecular differences from a divergence event of a known age. Because there are no published mutation rates for liverworts, this study applies an average cpDNA mutation rate derived from data on several land plant groups (e.g. Clegg et al., 1994; McDaniel and Shaw, 2003) to obtain crude estimates of past expansion in *J. colorata* populations.

Given an average rate of 1.43 x 10^{-8} substitutions/site/year, the two mismatch distribution expansion events are dated at approximately 16,900 and 101,400 year BP, with an error margin of around +/- 5,000 years. According to palaeoclimatic records, these dates fall within the last glacial period, which commenced at ~115,000 BP and terminated with the LGM at ~18,000 BP. The juxtaposition of the *trn*L-F mismatch distribution against the Vostok ice core record (figure 14) shows that these estimates generally correlate with past cooling trends in the southern hemisphere. In this case, expansion peaks would not align perfectly with minimum drops in temperature, because the genetic consequences of climate change are delayed. It should be noted that the purpose of this exercise is to simply explore methods of dating the expansion events and not to provide unequivocal evidence on the history of *J. colorata*.

4.5 Taxonomic Status of *J. oenops*

Studies on liverworts with inter-continentally disjunct, morphologically uniform sibling species (Wyatt, 1994) show that morphology and genetic diversity are not always correlated. Genetic structure also does not seem to determine ecological preferences (Bischler and Boisselier-Dubayle, 1997) as several cryptic and sibling species have been found living in close proximity (e.g. Dewey, 1989; Shaw, 2000). In this study, all four '*J. oenops*' specimens were found co-existing with *J. colorata*.

The position of the four '*J. oenops*' samples on the NJ tree confirms that they are genetically divergent from the *J. colorata* samples. Although very similar in appearance, a closer morphological assessment uncovered several differences between the '*J. oenops*' and *J. colorata* specimens. The leaf cuticle of '*J. oenops*' is

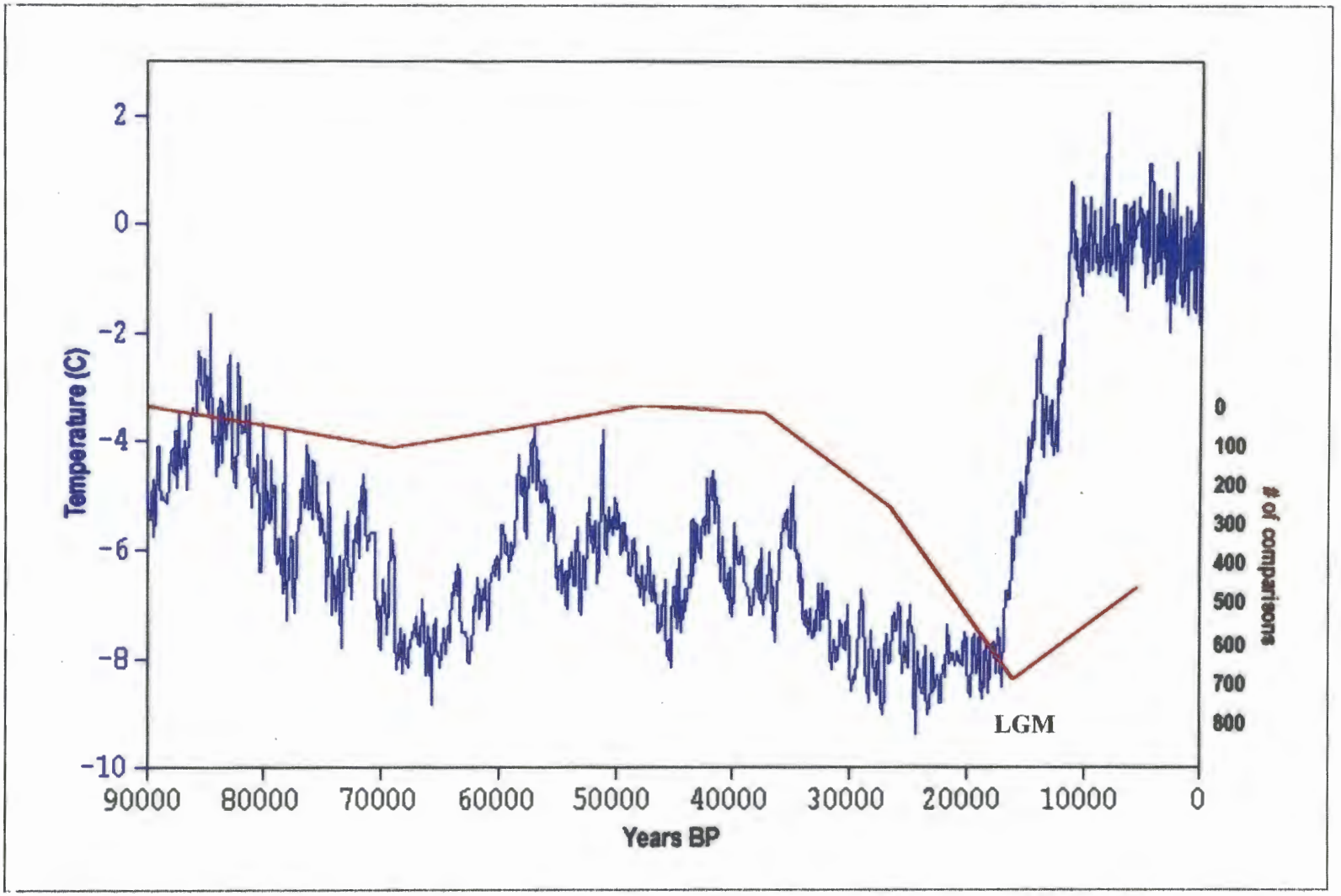

Figure 14. Overlay of expansion events interpreted from the *trn*L-F mismatch distribution onto the Vostok ice record of the last 90k years. The older expansion roughly lines up with a dip in temperature around 70k years BP, while the recent expansion event generally correlates with the LGM.

less papillose and the trigones are smaller compared to *J. colorata*. Also, the stem and leaves are smaller and the leaves are more incurved to the stem. These traits are found in a taxonomic description of *J. oenops* written before it was synonymised with *J. colorata* (Arnell, 1963).

Even though the *'J. oenops'* specimens seem to be genetically and morphologically distinct from *J. colorata*, the subject was not considered a main objective of this study because of the inherent limitations of a small sample size. Further work on many more specimens of *'J. oenops'* is needed before conclusions can be made regarding its taxonomic status.

4.6 Study Limitations

4.6.1 Sample Size

This study succeeded in sampling an adequate number of individuals per population across most of the recorded distribution within the Western Cape Province. Notable gaps indicated from the bioclimatic envelope and herbarium records occur within the Cederberg, Groot Winterhoek, Langeberg and Tsitsikamma mountain ranges.

Unfortunately, recorded populations in the Drakensberg were not sampled in this project because their distance from the Western Cape populations and high altitude habitats required more field time than was available. Future phylogeographical work on these populations would probably reveal an important chapter in the history of *J. colorata* and may provide insight on Afro-montane species distributions and diversity

across the subcontinent. Additionally, a global study of *J. colorata* populations would provide a broader phylogeographical context in which to interpret its evolutionary history in its entirety.

4.6.2 ITS Amplification

The inability to consistently amplify the nuclear ITS region of *J. colorata* samples because of fungal contamination resulted in a small dataset, which led to inconclusive results in most analyses. Liverwort-specific primers for ITS1 and ITS2 loci were developed and tested, but have so far failed to perform effectively. However, further adjustments of lab protocols are expected to result in a successful set of primers suitable for all leafy liverwort taxa.

4.6.3 Migration Rate Analysis

The size and similarity of the *trn*L-F sequence dataset hampered repeated attempts to accurately estimate migration rates among populations of *J. colorata*. The likelihood methods employed by the software packages LAMARC version 1.1 and MIGRATE version 1.7.3 (Beerli and Felsenstein, 1999) require a certain level of variation to heuristically calculate gene flow rates. It is thought that adding a second cpDNA region would resolve the analysis, but unfortunately additional laboratory work was not feasible in this short-term project.

4.6.4 GIS Data Scale

At present, a major limiting factor in constructing bioclimatic envelopes is the inadequate scale of digital data. Even with precise geographic co-ordinates of population localities, the large error distances of underlying parameter data (200 m error in the DEM layer used to derive slope, aspect and elevation) and the broad scale of the overlying GIS grids (1.5 km^2 for South African map) may lead to incorrect estimates. This is especially true for species like *J. colorata*, that live in small montane niches with particular microclimates that are unlikely to be detected in a broad-scale format. However, despite its shortcomings, the bioclimatic envelope of *J. colorata* does show a strong correlation with its recorded distribution and provides a general picture of favourable habitat in the Western Cape Province.

4.7 Conclusion

The presence of seven *trn*L-F cpDNA haplotypes in 11 populations shows that populations of *J. colorata* in the Western Cape are considerably diverse, with almost half of the genetic variation occurring among populations The nuclear ITS region results comprised five haplotypes in three populations, but showed no significant partitioning of diversity, due to the small sample size.

Phylogeographical inspection of the *trn*L-F haplotypes revealed that most of the diversity lies in southwestern Cape populations. The outlying populations to the east and north are dominated by the two most common haplotypes, indicating a relatively recent colonisation of that area. The NCA results lead to an inference of Restricted Gene Flow for most haplotypes and populations, except haplotype D, which was significant for Continuous Range Expansion. Mismatch distributions for both DNA regions indicate that at least two expansion events have occurred in *J. colorata* populations.

The combined population genetics, historical and demographic analyses in this study suggest that *J. colorata* populations have experienced repeated range shifts between refugial and expansion phases. These movements were influenced by major warming and cooling trends and variations in precipitation during the Quaternary period.

J. colorata populations probably retreated into upper montane refugia during the warming of the last interglacial period and the HA, and expanded into lower altitudes in response to the cooling trends of the last glacial period and the slightly cooler late

Holocene. Even with a broad understanding of the possible physical mechanisms involved, correlating discrete palaeoclimatic events to the evolutionary history of *J. colorata* has proven a difficult task. Without molecular clock rates for *J. colorata*, the timing of the expansion events can at best be estimated from mutation rates from other species. Rough estimates using an average cpDNA mutation rate places both expansions episodes within the last glacial period.

This study would be improved with further field and laboratory work. As indicated in the GIS bioclimatic envelope, there are sampling gaps across the distribution of *J. colorata* that may reveal additional phylogeographical evidence. Incorporating the Drakensberg populations as well as several global populations would expand the scale of historical inference. Efforts to augment the nuclear ITS dataset would be equally worthwhile, in order to corroborate or refute the history inferred from the *trn*L-F sequence data and to permit accurate gene flow analyses.

References

Arbogast, B. S., and Kenagy, G. J. (2001). Comparative phylogeography as an integrative approach to historical biogeography. *Journal of Biogeography* **28,** 819-825.

Arnell, S. (1963). "Hepaticae of South Africa." Stockholm.

Avery, D. M. (1993). Last interglacial and Holocene Altithermal environments in South Africa and Namibia: micromammalian evidence. *Palaeogeography, Palaeoclimatology, Palaeoecology* **101,** 221-228.

Avise, J. C. (1998). The history and purview of phylogeography: a personal reflection. *Molecular Ecology* **7,** 371-379.

Avise, J. C. (2000). "Phylogeography: the history and formation of species." Harvard University Press, Cambridge, Mass.

Baxter, A. (1989). "Pollen analysis of a Table Mountain cave deposit." Unpublished BSc (Hons) thesis, University of Cape Town.

Beerli, P., and Felsenstein, J. (1999). Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. *Genetics* **152,** 763-773.

Bermingham, E., and Mortiz, C. (1998). Comparative phylogeography: concepts and applications. *Molecular Ecology* **7,** 367-369.

Bischler, H., and Boisselier-Dubayle, M. C. (1997). Population genetics and variation in liverworts. *Advances in Bryology* **6,** 1-34.

Bush, A. B. G., and Philander, S. G. H. (1999). The climate of the Last Glacial Maximum: Results from a coupled atmosphere-ocean general circulation model. *Journal of Geophysical Research-Atmospheres* **104,** 24509-24525.

Clegg, M. T., Gaut, B. S., Learn, G. H., and Morton, B. R. (1994). Rates and patterns of chlororplast DNA evolution. *Proceedings of the National Academy of Science* **91,** 6795-6801.

Clement, M., Posada, D., and Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9,** 1657-1660.

Cockcroft, M. J., Wilkinson, M. J., and Tyson, P. D. (1987). The application of a present-day climatic model to the late quaternary in southern Africa. *Climate Change* **10,** 161-181.

Cottrell, J. E., Munro, R. C., Tabbener, H. E., Gillies, A. C. M., Forrest, G. I., Deans, J. D., and Lowe, A. J. (2002). Distribution of chloroplast DNA variation in British oaks (*Quercus robur* and *Q. petraea*): the influence of postglacial colonisation and human management. *Forest Ecology and Management* **156.**

Cowling, R. M. (1987). Fire and its role in coexistence and speciation in Gondwanan shrublands. *South African Journal of Science* **83,** 106-112.

Cowling, R. M., Holmes, P. M., and Rebelo, A. G. (1992). Plant diversity and endemism. *In* "The Ecology of Fynbos: Nutrients, Fire and Diversity." (R. M. Cowling, Ed.), pp. 62-112. Oxford University Press, Cape Town.

Cruzan, M. B., and Templeton, A. R. (2000). Paleoecology and coalescence: phylogeographic analysis of hypotheses from the fossil record. *TRENDS in Ecology and Evolution* **15,** 491-496.

Deacon, H. J., and Lancaster, N. (1988). "Late Quaternary palaeoenvironments of southern Africa." Clarendon Press, Oxford.

Deacon, H. J., Jury, M. R., and Ellis, F. (1992). Selective Regime and Time. *In* "Fynbos: Nutrients, Fire and Diversity." (R. M. Cowling, Ed.). Oxford University Press, Cape Town.

deMenocal, P. B. (1995). Plio-Pleistocene African climate. *Science* **270,** 53-59.
Dewey, R. M. (1989). Genetic variation in the livewort *Riccia dictyospora* (Ricciaceae, Hepaticopsida). *Systematic Botany* **14,** 155-167.
Doyle, J. J., and Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19,** 11-15.
Dumolin-Lapegue, S., Demesure, B., Fineschi, S., Le Corre, V., and Petit, R. J. (1997). Phylogeographic Structure of White Oaks Throughout the European Continent. *Genetics* **146,** 1472-1487.
During, H. J. (2000). Life history characteristics of threatened bryophytes. *Z. Okologie u. Naturschutz* **9,** 19-26.
Ellstrand, N. C., and Elam, D. R. (1993). Population Genetic Consequences of Small Population Size: Implications for Plant Conservation. *Annual Review of Ecology and Systematics* **24,** 217-242.
Emerson, B. C., Paradis, E., and Thebaud, C. (2001). Revealing the demographic histories of species using DNA sequences. *TRENDS in Ecology and Evolution* **16,** 707-716.
Excoffier, L., Smouse, P. E., and Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131,** 479-491.
Frahm, J. P. (2000). New and interesting mosses from Baltic and Saxonian amber. *Lindbergia* **25**.
Gradstein, S. R., Churchill, S. P., and Salazar-Allen, N. (2001). Guide to the Bryophytes of Tropical America. *In* "Memoirs of the New York Botanical Garden." pp. 2-12. New York Botanical Garden Press, New York.
Grolle, R. (1971). Jamesoniella und Verwandte. *Feddes Repertorium* **82,** 1-99.
Grolle, R., and Long, D. G. (2000). An annotated checklist of the Hepaticae and Anthrocerotae of Europe and Macaronesia. *Journal of Bryology* **22,** 103-140.
Guiot, J., Pons, A., Beaulieu, J.-L. d., and Reille, M. (1989). A 140,000-year continental climatic reconstruction from two European pollen records. *Nature* **338,** 309-313.
Hamby. (1988).
Harding, R. M. (1996). New phylogenies: an introductory look at the coalescent. *In* "New Uses for New Phylogenies." (P. H. Harver, A. J. L. Brown, J. M. Smith, and S. Nee, Eds.), pp. 15-22. Oxford University Press, Oxford.
Hare, M. P. (2001). Prospects for nuclear gene phylogeography. *TRENDS in Ecology and Evolution* **16,** 700-706.
Hewitt, G. M. (1999). Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* **68,** 87-112.
Hewitt, G. M. (2000). The genetic legacy of the Quaternary ice ages. *Nature* **405,** 907-913.
Hudson, R. R. (1990). Gene genealogies and the coalescent process. *In* "Oxford Surveys in Evolutionary Biology." (Futuyama, and J. D. Antonovics, Eds.), pp. 1-44. Oxford University Press, New York.
Iriondo, M. H. (1999). Last Glacial Maximum and Hypsithermal in the Southern Hemisphere. *Quaternary International* **62,** 11-19.
Knowles, L., and Maddison, W. P. (2002). Statistical Phylogeography. *Molecular Ecology* **11,** 2623-2635.
Kukla, G. J. (1989). Long continental records of climate: an introduction. *Palaeogeography, Palaeoclimatology, Palaeoecology* **72,** 1-9.

Laaka-Linberg, S., Hedderson, T. A., and Longton, R. E. (2000). Rarity and reproductive characters in British hepatic flora. *Lindbergia* **25,** 78-84.

Linder, H. P. (2003). The radiation of the Cape flora, southern Africa. *Biological Review* **78,** 597-638.

McDaniel, S. F., and Shaw, A. J. (2003). Phylogeographic structure and cryptic speciation in the trans-antarctic moss *Pyrrhorbryum mnioides*. *Evolution* **57,** 205-215.

Meadows, M. E., and Sugden, J. M. (1991). A vegetation history of the last 14,000 years on the Cederberg, southwestern Cape Province. *South African Journal of Science* **87,** 34-43.

Meadows, M. E., and Baxter, A. J. (1999). Late Quaternary paleoenvironments of the southwestern Cape, South Africa: a regional synthesis. *Quaternary International* **57/58,** 193-206.

Meadows, M. E., and Baxter, A. J. (2001). Holocene vegetation history and palaeoenvironments at Klaarfontein Springs, Western Cape, South Africa. *Holocene* **11,** 699-706.

Midgley, G. F., Hannah, L., Roberts, R., MacDonald, D. J., and Allsopp, J. (2001). Have Pleistocene climatic cycles influences species richness patterns in the greater Cape Mediterranean Region? *Journal of Mediterranean Ecology* **2,** 137-144.

Midgley, G. F., Hannah, L., Millar, D., Thuiller, W., and Booth, A. (2003). Developing regional and species-level assessments of climate change impacts on biodiversity in the Cape Floristic Region. *Biological Conservation* **12,** 87-97.

Parkington, J., Cartwright, C., Cowling, R. M., Baxter, A., and Meadows, M. E. (2000). Palaeovegetation at the last glacial maximum in the western Cape, South Africa: wood charcoal and pollen evidence from Elands Bay Cave. *South African Journal of Science* **96,** 543-546.

Partridge, T. C., Avery, D. M., Botha, G. A., Brink, J. S., Deacon, J., Herbert, R. S., Maud, R. R., Scholtz, A., Scott, L., Talma, A. S., and Vogel, J. C. (1990). Late Pleistocene and Holocene Climatic-Change in Southern Africa. *South African Journal of Science* **86,** 302-306.

Partridge, T. C., Scott, L., and Hamilton, J. E. (1999). Synthetic reconstructions of southern African environments during the Last Glacial Maximum (21-18 kyr) and the Holocene Altithermal (8-6 kyr). *Quaternary International* **57-8,** 207-214.

Petit, J. R., Jouzel, J., Raynaud, D., Barkov, N. I., Barnola, J. M., Basile, I., Bender, M., Chappellaz, J., Davis, M., Delaygue, G., Delmotte, M., Kotlyakov, V. M., Legrand, M., Lipenkov, V. Y., Lorius, C., Pepin, L., Ritz, C., Saltzman, E., and Stievenard, M. (1999). Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* **399,** 429-436.

Ponel, P. (1995). Rissian, Eemian, and Wurmian Coleoptera assemblages from La Grande Pile (Vosges, France). *Palaeogeography, Palaeoclimatology, Palaeoecology* **114,** 1-41.

Posada, D., Crandall, K. A., and Templeton, A. R. (2000). GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. *Molecular Ecology* **9,** 487-488.

Read, D. J., Duckett, J. G., Francis, R., Ligrone, R., and Russell, A. (2000). Symbiotic fungal associations in 'lower' land plants. *Philosophical Transactions of the Royal Society of London Series B - Biological Sciences* **355,** 815-830.

Richardson, J. E., Weitz, F. M., Fay, M. F., Cronk, Q. C. B., Linder, H. P., Reeves, G., and Chase, M. W. (2001). Rapid and recent origin of species richness in the Cape flora of South Africa. *Nature* **412,** 181-183.

Rogers, A. R., and Harpending, H. (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution* **9,** 552-569.

Schaal, B. A., Hayworth, D. A., Olsen, K. M., Rauscher, J. T., and Smith, W. A. (1998). Phylogeographic studies in plants: problems and prospects. *Molecular Ecology* **7**.

Schaal, B. A., and Olsen, K. M. (2000). Gene genealogies and population variation in plants. *Proceedings of the National Academy of Science* **97,** 7024-7029.

Schneider, S., and Excoffier, L. (1999). Estmation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics* **152,** 1079-1089.

Schneider, S., Roessli, D., and Excoffier, L. (2000). Arlequin ver. 2.000: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

Scholtz, A. (1986). "Palynological and Palaeobotanical Studies in the Southern Cape." Unpublished MA thesis, University of Stellenbosch.

Schulze, R. E. (1997). South African Atlas of Agrohydrology and -Climatology. Water Research Commission, Pretoria.

Schuster, R. M. (1966). "The Hepaticae and Anthocerotae of North America." Columbia University Press, New York.

Schuster, R. M. (2002). "Austral Hepaticae, Part II." Berlin.

Scott, L. (1993). Palynological Evidence for Late Quaternary Warming Episodes in Southern Africa. *Palaeogeography, Palaeoclimatology, Palaeoecology* **101,** 229-235.

Scott, L. (1994). Past vegetation changes in mountainous areas South Africa revealed by pollen analysis of Hydrax middens. *In* "XIIIth Plenary Meeting AETFAT." pp. 1007-1014, Malawi.

Shaw, A. J. (2000). Molecular phylogeography and cryptic speciation in the mosses, *Mielichhoferia elongata* and *M. mielichhoferiana* (Bryaceae). *Molecular Ecology* **9,** 595-608.

Shaw, A. J., Cymon, J. C., and Boles, S. B. (2003). Global patterns in peatmoss biodiversity. *Molecular Ecology* **12,** 2553-2570.

Soltis, D. E., Gitzendanner, M. A., Strenge, D. D., and Soltis, P. S. (1997). Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. *Plant Systematic Evolution* **206,** 353-373.

Stute, M., and Talma, A. S. (1997). Glacial temperatures and moisture transport regimes reconstructed from noble gases and 18O, Stampriet Aquifer, Namibia. *In* "Isotopic techniques in the study of environmental change." pp. 307-318. International Atomic Energy Agency, Vienna.

Swofford, D. L. (1998). PAUP* : Phylogenetic Analysis Using Parsimony. Sinauer Associates, Sunderland, MA.

Taberlet , L. G., Pautou, G., and Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17,** 1105-1109.

Templeton, A. R., and Sing, C. F. (1993). A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping.

IV. Nested analysis with cladogram uncertainty and recombination. *Genetics* **134,** 659-669.

Templeton, A. R., Routman, E., and Phillips, C. A. (1995). Separating Population Structure from Population History: A Cladistic Analysis of the Geographical Distribution of Mitochondrial DNA Haplotypes in the Tiger Salamander, *Ambystoma tigrinum*. *Genetics* **140**.

Templeton, A. R. (1998). Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology* **7,** 381-397.

Tyson, P. D. (1986). "Climatic Change and Variability in Southern Africa." Oxford University Press, Cape Town.

Tyson, P. D. (1999). Atmospheric circulation changes and palaoclimates of southern Africa. *South African Journal of Science* **95,** 194-201.

Tyson, P. D., Odada, E. O., and Partridge, T. C. (2001). Late Quaternary environmental change in southern Africa. *South African Journal of Science* **97,** 139-150.

Verheyen, E., Salzburger, W., Snoeks, J., and Meyer, A. (2003). Origin of the superflock of cichlid fishes from Lake Victoria, East Africa. *Science* **300,** 325-329.

Walther, G., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., Fromentin, J., Hoegh-Guldbergh, O., and Bairlein, F. (2002). Ecological responses to recent climate change. *Nature* **416,** 389-395.

White, T. J., Bruns, T., Lee, S., and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* "PCR Protocols: A Guide to Methods and Applications." (M. Innis, P. Gelfand, J. Sninsky, and T. White, Eds.), pp. 315-322. Academic Press, San Diego.

Widmer, A., and Lexer, C. (2001). Glacial refugia: sanctuaries for allelic richness, but not for gene diversity. *TRENDS in Ecology and Evolution* **16,** 267-269.

Wyatt, R. (1994). Population genetics of bryophytes in relation to their reproductive biology. *Journal of the Hattori Botanical Laboratory* **76,** 147-157.